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BEARING ON HEREDITY AND
VARIATION

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GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

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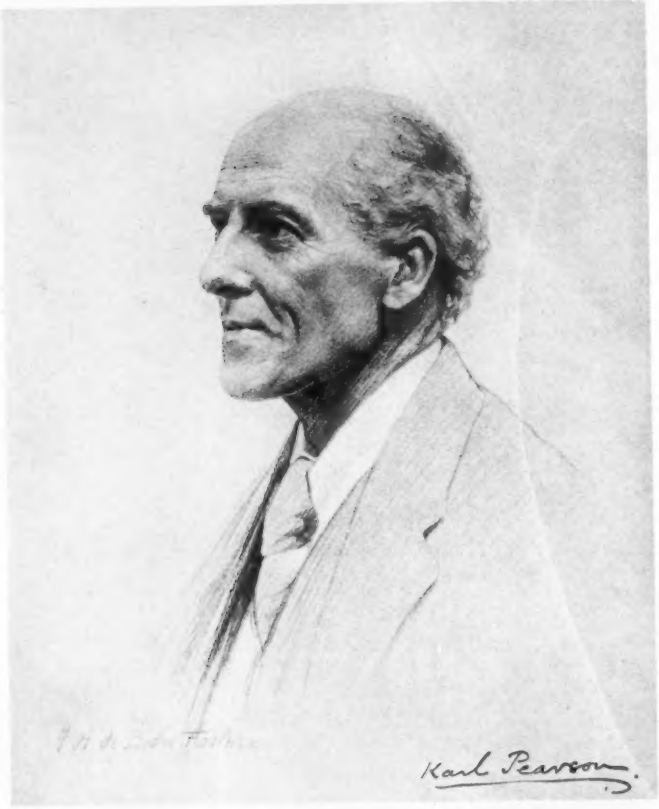
Karl Pearson.

MUTANTS IN HABROBRACON

P. W. WHITING

University of Pittsburgh, Pittsburgh, Pennsylvania

Received April 8, 1931

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INTRODUCTION

The parasitic wasp, Habrobracon, is excellent material for studying the time of occurrence of mutations producing visible effects, since the haploid nature of the males prevents recessives from being carried over more than one generation before the mutant individuals appear. Recessive mutations occurring in the germ tract of the male will become evident in half the sons of the females arising from eggs fertilized by sperm bearing the mutant gene. Recessives occurring in the germ tract of the female will appear in her mutant sons. Should the mutated eggs be fertilized, her daughters developing from these eggs will produce sons of which fifty percent will show the new trait. Exceptions may, of course, appear, due to simultaneous occurrence of lethals or low viability reducing proportions of mutants below expectation, but even in this case count of a fair number of male offspring should bring the mutation to light.

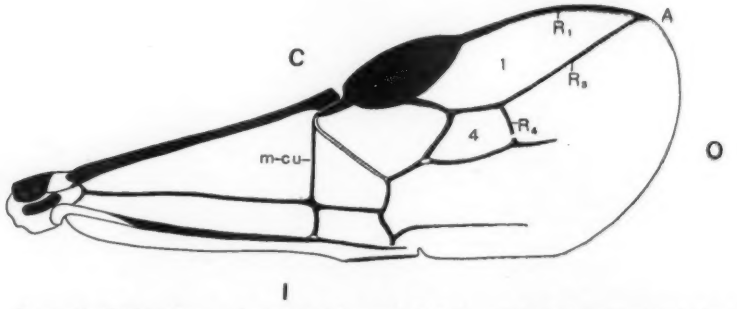


FIGURE 1—Right primary wing. C. Costal margin. O. Outer margin. I. Inner margin. A. Apex. R_1, R_3, R_4. First, third and fourth branches of radius vein. m-cu. Medio-cubital cross-vein. 1, 4. First and fourth radial cells.

The purpose of the present paper is to put on record the salient points in regard to the occurrence of the mutants thus far found, together with a description of their traits. Details of inheritance including linkage relations will not be considered here. All factors are inherited in sex-linkoid fashion as is to be expected in species producing males by haploid parthenogenesis (arrenotoky).

THE MUTANT TYPES

Defective, d_{11vs} (venation)

One of the commonest hereditary variations occurring in nature is a greater or less tendency toward breaks in the fourth branch of the radius vein, R_4 (figure 1). There are multiple genetic factors involved and tem-

perature influences the trait, the greater defect being correlated with higher temperature. A single gene occurring early in the work, possibly derived from wild stock (WHITING 1924), was isolated and found to give about 95 percent phenotypically defective under standard conditions, 30°C. Defective is of normal viability and fertility.

Wrinkled, w (wings)

A female from pedigree cultures carried a mutant factor causing difficulty in shedding pupal integument (WHITING 1926b). As a consequence irregular wrinkling of the wings results and portions of this integument are found adhering to the antennae which show a tendency to fragment. The fraternity segregating the mutants was small, consisting of males, type 6, wrinkled 7, and females, type 4. Failure of the character to appear among the mother's siblings indicates that the mutation arose late in spermatogenesis or in ovogenesis of one of the grandparents of the mutants or in the germ tract of the mother. Wrinkled is fertile in both sexes but is mechanically hampered in eclosion and in other activities.

Reduced, r (wings and venation)

ACHSA M. BEAN found in March, 1925 (WHITING 1926b), a single male with small wings and irregular reduced venation (plate 1, figures 18–21). The character did not appear in any of the sibs and hence the mutation must have occurred late in ovogenesis. The trait is variable but always distinct from type. Reduced affects chiefly the middle area of the wings and usually causes disappearance of vein m-cu (figure 1). It is of good viability and fertility in both sexes.

Lethal naked pupae

A single female in pedigree culture proved heterozygous for a factor causing metamorphosis of larvae into small sized naked pupae which attained approximately normal coloration before death. This factor (WHITING 1921b) proved to be linked with orange (19.5 percent crossovers).

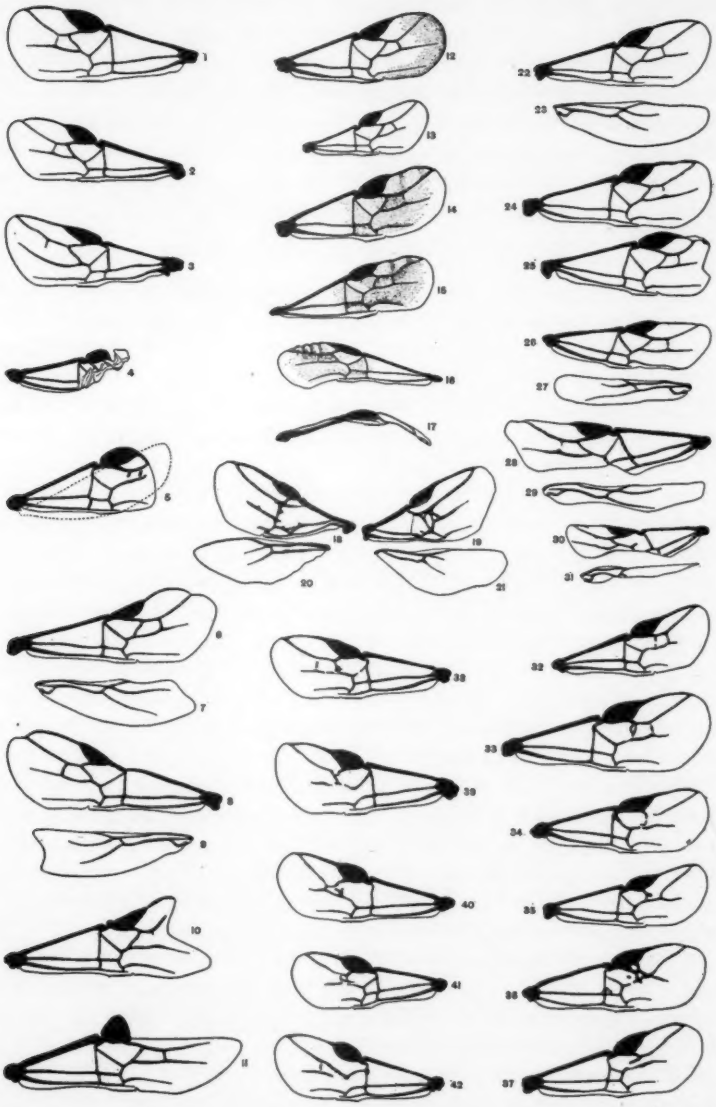
Sooty, s_{II} (mesosternum)

Variation in general body pigmentation is controlled largely by temperature but the influence of hereditary factors is also obvious. When reared at 30°C the wasps show differences in color of the mesosternum. A major factor for sooty, s_{II}, was shown (WHITING 1926a) to be linked with d_{II95}, but percentage of crossovers is uncertain due to overlaps. Minor factors for the same trait, independently segregating, were also found.

LEGEND FOR PLATE 1

Wings of various mutant types. All shown from dorsal side except figure 17 which is viewed from costal margin. Magnification 13½×.

FIGURE 1.—Semilong.
FIGURE 2.—Fused.
FIGURE 3.—Beaded.
FIGURE 4.—Unexpanded.
FIGURE 5.—Truncate with outline of secondary.
FIGURES 6–11.—Notched, from four individuals.
FIGURE 12.—Long.
FIGURE 13.—Miniature.
FIGURES 14–17.—Wavy.
FIGURES 18–21.—Reduced, from one individual.
FIGURES 22–23.—Type.
FIGURES 24–25.—Confluent.
FIGURES 26–31.—Narrow, from three individuals.
FIGURES 32–42.—Shot.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Deficiency (antennae and posterior extremity)

Failure of normal development of structures at anterior or at posterior extremities sometimes occurs. Antennal segments may be irregularly fused. External genitalia may be small or lacking. Digestive tract may fail to open posteriorly. No definite factors were isolated for these deficiencies which were shown to be controlled by heredity and to be highly correlated with age of mothers (WHITING 1926c). Many of the genes cited in the present paper cause greater or less tendency toward antennal deficiency.

Orange, o (eye), and its four allelomorphs

A quadruple allelomorphic series of factors affecting eye color was reported (WHITING and BURTON 1926). The normal wild-type, jet black eyes and dark brown ocelli, mutated to orange, *o*, in which the ocelli have but a slight trace of pigment while the eyes are orange varying to pink and red. The first appearance of orange (WHITING 1921a) was in a fraternity of 254 males, a single mutant found March 27, 1920. The mother was less than seven days of age at the time the egg was laid. A second orange mutant has been recorded (WHITING 1921a, p. 48) as a single male in a fraternity of type stock including also 115 type males and 83 type females.

On June 24, 1924, there appeared in a fraternity of orange stock 4 ivory, o^i, eyed males. Eleven orange males and 22 orange females had been produced in vial a. Vial b showed 12 orange males, 9 orange females of which 6 when tested produced only orange, and the 4 ivory mutants. There were therefore produced by the mother at least 29 orange-bearing eggs besides the 4 ivory. The mutation probably occurred in ovogenesis previous to maturation. Ivory has colorless ocelli and compound eyes of a greenish white with occasionally a trace of pink. A second mutation to ivory is reported by EDWARD J. WENSTRUP. A female from orange stock (number 3) crossed with a type male (9B3) produced orange males 34, type females 113, and 1 orange female. This orange daughter produced 38 orange males and 34 ivory (checked by breeding). The mutation therefore took place in the sperm, changing the normal allelomorph directly to ivory.

In January, 1925, there appeared, from a cross of ivory female by type male, a single female with light ocelli but with the compound eyes black as in type. There were 34 of the expected type female sibs. This mutant proved to be a compound for ivory and hence the mutation took place in spermatogenesis involving at least one sperm. Light, o^l, has black eyes but ocelli are very light in compound with ivory or with orange. In pure light stock the ocelli may be characterized as light brown, somewhat darker in

the males. If males are reared at low temperature it is often difficult to distinguish them from type. Light has been discarded.

A fifth allelomorph in the orange locus appeared December 1, 1929 (132.7b MMT). MAGNHILD M. TORVIK found from a cross of an ivory female by a type male a single female with light ocelli and eyes very dark reddish brown. There were 11 type sisters and 7 ivory brothers in the same vial. There were 35 type sisters, one type brother (biparental) and 39 ivory brothers in the total fraternity. This mutant, called dahlia, o^d, proved compound for ivory indicating that the mutation had occurred in spermatogenesis changing the normal allelomorph. Dahlia-ivory or dahlia-orange compound females are lighter in color than pure dahlia males or females.

The locus orange appears to be especially unstable but there is certainly no striking increase of mutability correlated with X-ray treatment. As has been previously reported (WHITING 1928) there is a very high correlation between mosaicism and apparent mutation for this locus. New data on mutation and mosaicism will be reported elsewhere.

Cantaloup, c (eyes)

A female from orange stock was treated March, 1928 (filter, ½ mm aluminum; peak voltage, 45 K. V.; milliamperes, 8; distance, 15 cm; duration, 15 minutes; dosage, about 1100 R), by P. W. WHITING and produced (vial a) 5 orange males and 1 male with eyes resembling ivory. Crosses with ivory females resulted in 328 ivory males, 10 orange males (biparental), and 193 orange females. By subsequent tests with type it was shown that the mutant was a double recessive, orange cantaloup. Cantaloup varies considerably, ranging from creamy white at eclosion through pale pink and light orange and finally deep red. In mixed culture it may be confused with ivory or with orange.

Maroon, m_a (eyes)

In January, 1931, L. H. BENKERT found a male with light ocelli and maroon eyes (199.18 LHB). There were but two other males in the fraternity, both type, so that the time of occurrence of mutation cannot be determined. Maroon is deep reddish brown, somewhat resembling dahlia.

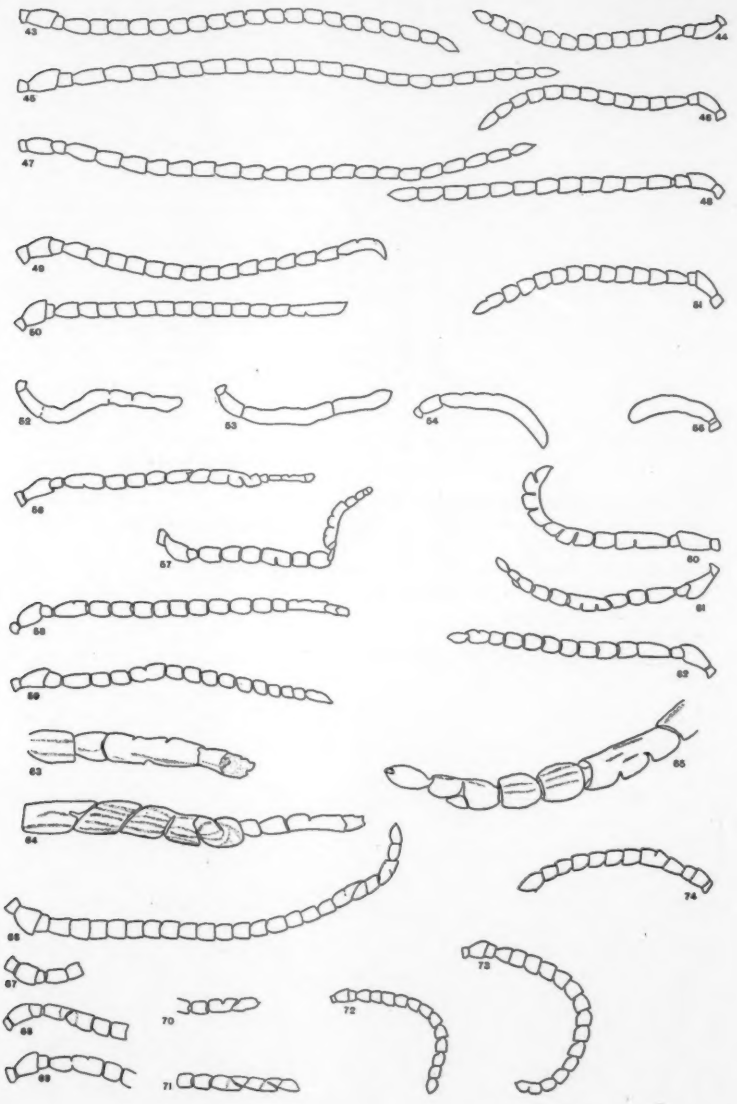
Minnesota yellow, M_y (base of antennae)

Stock sent by ROYAL N. CHAPMAN from Minneapolis, August, 1929, included wasps with basal three or four segments of antennae decidedly yellow when reared at 30°C. The difference evidently depends upon one gene with various modifiers, determining the extent of the trait which may

LEGEND FOR PLATE 2

Antennae of various mutant types. Antennae of males are shown as originating at left, of females at right. Magnification 33⅓× except 63, 64, and 65 which are 80×.

FIGURES 43–44.—Type.
FIGURES 45–46.—Semilong.
FIGURES 47–48.—Long.
FIGURES 49–51.—Confluent.
FIGURES 52–55.—Fused.
FIGURES 56–65.—Tapering.
FIGURES 66–71.—Beaded.
FIGURES 72–73.—Miniature.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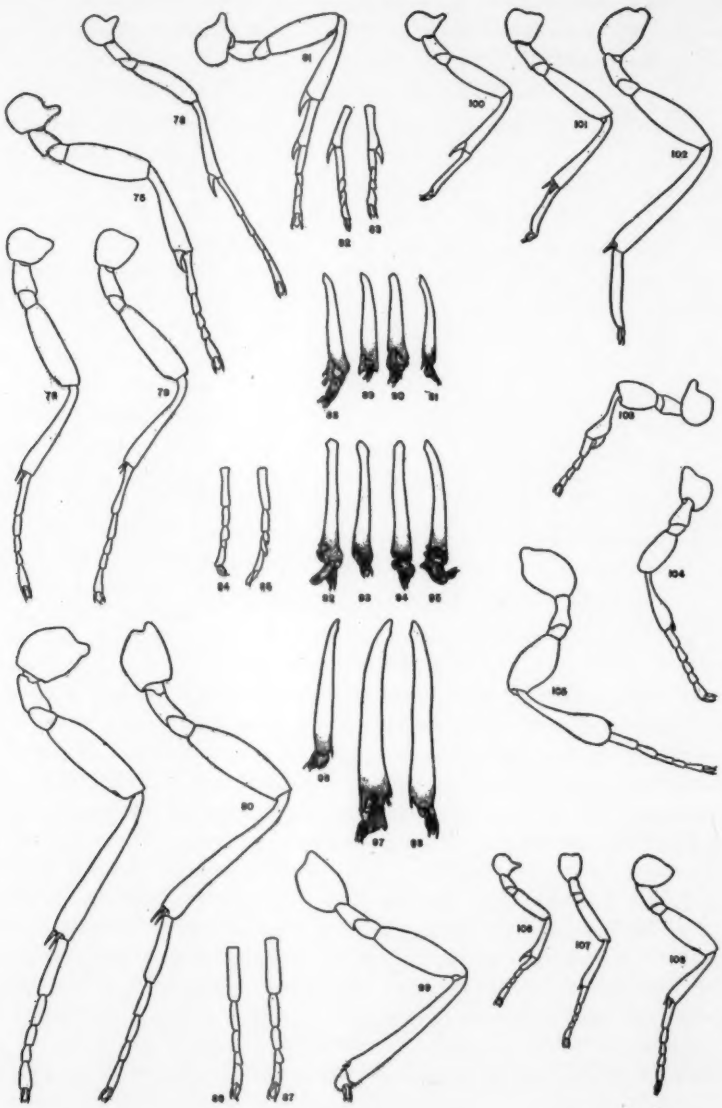
LEGEND FOR PLATE 3

Legs of various mutant types. Magnification 33⅓×.
FIGURES 75–77.—Prothoracic, mesothoracic, and metathoracic legs of type.
FIGURES 78–80.—Long.
FIGURES 81–83, 84–85, 86–87.—Female heterozygous for stumpy.
FIGURES 88–91, 92–95, 96–99.—Stumpy male.
FIGURES 100–102.—Fused.
FIGURES 103–105.—Beaded.
FIGURES 106–108.—Miniature.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be increased by selection. Minnesota yellow is of normal viability and may be regarded as dominant since heterozygous females show the trait under standard conditions.

Yellow, Y (base of antennae)

On February 17, 1929, ANNA R. WHITING found two mutant males, one in vial b and one in vial c of a fraternity (10.6 ARW) containing 82 normal brothers. The mother had been treated (filter, 1 mm aluminum; peak voltage, 50 K. V.; milliamperes, 8; distance, 15 cm; duration, 20 minutes; dosage, about 1460 R) as a four day old larva. The mutants had the basal three segments of the antennae strikingly yellow. It is possible that sibs bearing the gene may have been present, but this is very unlikely as the culture was reared under standard conditions and all individuals were carefully examined. The yellow color is more definitely confined to the three basal segments in this type than is the case in Minnesota yellow, but lowering of temperature during development induces melanogenesis in both cases. Since heterozygous females show the trait, it may be called dominant. Yellow is fertile in both sexes but of less viability than type. Distal segments of antennae often appear pale and if temperature is but slightly increased over standard, yellow wasps become deformed and often die in cocoons. This is not the case with Minnesota yellow in which viability is normal. The linkage relations of the two types of yellow are also different.

Beaded, b (legs)

In August, 1929, ANNA R. WHITING found a fraternity of males containing a number of mutants with beaded legs (plate 3, figures 103–105). The mother was among a miscellaneous lot of larvae treated July 31 (filter, 1 mm aluminum; peak voltage, 50 K. V.; milliamperes, 8; distance, 15 cm; duration, 35 minutes; dosage, uncertain). Since she was set August 8, she had probably been in the embryo or young larval condition at time of treatment. In vial a there was found but 1 wasp, beaded, and in vial b, 18 beaded to 8 normal sibs. The excess of mutants over equality expected if the entire gonad were heterozygous is not significant. The proportions of the various parts of the wing of beaded are very aberrant (plate 1, figure 3) and wings often fail to expand. Antennae are very likely to show fusion of segments and often more or less distal deficiency (plate 2, figures 66–71). A large and variable percentage of beaded fail to eclose. Males of beaded are fertile but viable females have not been found.

Stumpy, s_t (legs)

On September 3, 1930, ANNA R. WHITING found a single male with

stumpy legs (223.1 g ARW) among 36 normal male sibs in vial g. There were 186 normal male sibs in the entire fraternity. The mother had been rayed as a one or two day old larva (filter, ½ mm aluminum; peak voltage, 60 K. V.; milliamperes, 5; distance, 15 cm; duration, 40 minutes; dosage 3250 R units). The most striking characteristic of stumpy is extreme reduction of tarsal segments (plate 3, figures 88–99). Appendages appear otherwise normal. Stumpy may be called a recessive but a large proportion of heterozygous females bear a spur at the tip of their prothoracic metatarsi (plate 3, figures 81–83). More or less irregularity obtains in tarsal segments of all legs (plate 3, figures 84–87) so that heterozygotes are not as sure footed as pure type. No metatarsal spur has ever been found on middle or hind legs of heterozygotes nor has a prothoracic metatarsal spur ever been found in type females. This spur closely resembles the spur normally occurring at the end of the prothoracic tibia. It may be present on either or both front legs, but many heterozygotes fail to show it. Even in its absence it is usually possible to identify heterozygotes by tarsal irregularity. Stumpy is of normal viability and fertility except that females have difficulty in feeding from caterpillars and consequently may die without producing eggs. Apparently they cannot brace themselves well enough to insert their sting for puncturing in order to feed.

Miniature, m, (body)

A male of type stock (number 11) was treated in March 1928 (filter, ½ mm aluminum; peak voltage, 45 K. V.; milliamperes, 8; distance, 15 cm; duration, 40 minutes; dosage about 2950R), by P. W. WHITING and mated on the same day and on five succeeding days. One of the daughters from the last mating produced 9 type males, 6 miniature males, and 44 type females. Miniature is in general smaller than type. The primary wing (plate 1, figure 13) is less rounded on the costal margin. The antennae are shortened, both by shortening of the segments and by reduction of their number (plate 2, figures 72 and 73). There is also more irregularity in the way the segments are set together. No marked disproportion appears in the reduction of the size of the legs (plate 3, figures 106–108). Miniature is a semi-lethal. Many die as pupae. Adult males are of good fertility but adult females are almost sterile.

Short, s_h (wings)

Cultures derived from some of the sibs of the miniature mutants contained wasps with very short wings. It is uncertain whether short occurred as a mutation at approximately the time of the mutation to miniature or

LEGEND FOR PLATE 4

FIGURES 109–120.—Magnification 33½×; 121–131, 13½×.

FIGURES 109–110, 111–112, 113–116.—Prothoracic, mesothoracic, and metathoracic legs of twisted. Figures 115–116 are from one individual.

FIGURES 117, 118, 119–120.—Same of constricted.

FIGURE 121.—Dorsal view, thorax of type.

FIGURE 122.—Dorsal view, thorax of broad.

FIGURE 123.—Dorsal view, thorax and wing bases of broad showing reduplication of right secondary.

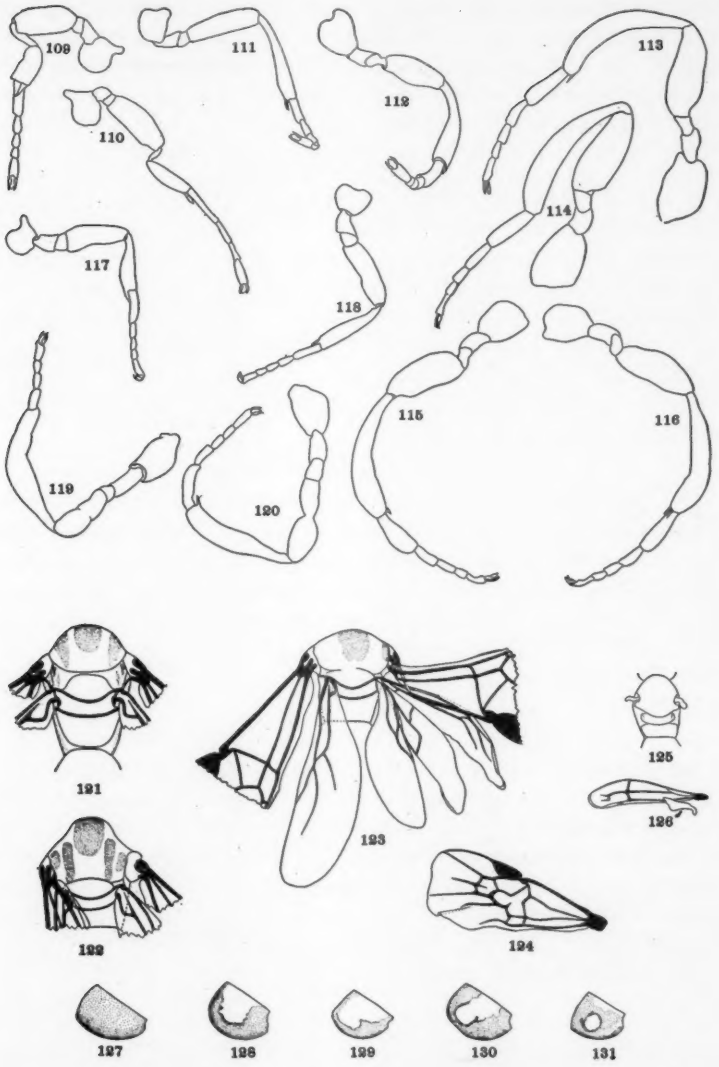
FIGURE 124.—Primary of a variant of broad showing tendency toward reduplication.

FIGURE 125.—Dorsal view, thorax of vestigial.

FIGURE 126.—Wing of vestigial unusually well developed for this type.

FIGURE 127.—Left view, mesopleuron of type.

FIGURE 128–131.—Left views, mesopleura of spread.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whether it had been carried in the stock. Short is very susceptible to temperature, overlapping with normal to a considerable extent. It is impossible to distinguish a culture of short from type when reared at room temperature. Under standard conditions, 30°C, wings of short average much smaller, but even in this case there is more or less overlapping. No correlated differences have been found.

Twisted, t_w (legs)

A male of type stock (number 11) was treated January 11, 1930 (filter, ½ mm aluminum; peak voltage, 45 K. V.; milliamperes, 8; distance, 15 cm; duration, 40 minutes; dosage, 3560 R units) by R. ELIZABETH CHALMERS and mated to four females (number 11) (January 11, 12, 13, and 17). One of the daughters from the last mating produced 35 type males and 14 males with twisted legs. Legs of twisted show much malformation and irregular bending (plate 4, figures 109–116). Many individuals fail to eclose, probably on account of mechanical difficulties. Males are of normal fertility. Females are very weak.

Constricted, C_s (femora)

In August, 1930, L. H. BENKERT found a single female with constricted femora (192.18b). There were 8 sisters in vial a, 9 in b including the mutant, 10 in c and 9 in d. Constricted is a dominant and apparently lethal in the male. It is very difficult to obtain offspring from constricted females and fraternities are small. The difficulty is probably mechanical and concerned with feeding from the caterpillars. The trait appears in all three pairs of legs (plate 4, figures 117–120) but is most obvious in the metathoracic.

Narrow, n (wings)

A male of type stock (number 11) was treated April 25, 1930 (filter, ½ mm aluminum; peak voltage, 45 K. V.; milliamperes, 8; distance, 15 cm; duration, 25 minutes; dosage, 2100 R units) by R. ELIZABETH CHALMERS and mated to three females (number 11) (April 25, 28, 31). One of the daughters from the last mating produced 32 type males and 14 males with narrow wings. Narrow (plate 1, figures 26–31) is somewhat variable but does not overlap with type. It is of good viability and fertility in the male. Females are apparently sterile.

Wavy, w_a (wings)

A male of type stock (number 11) was treated June 30, 1930 (filter, ½ mm aluminum; peak voltage, 45 K. V.; milliamperes, 8; distance, 15 cm; duration, 25 minutes; dosage, 2740 R units) by R. ELIZABETH CHALMERS and

mated to four females (number 11) (July 7, 10, 17, 24). Four daughters among 11 tested from this last mating produced type males and males with wavy wings as follows: type 6, wavy 7; type 14, wavy 11; type 7, wavy 6; type 8, wavy 5. The other seven daughters produced only type. Wings of wavy (plate 1, figures 14–17) are shortened, showing transverse waves which are especially noticeable near the distal part of the costal margin. Antennae are usually normal but in many cases one or both become markedly depigmented distally, the segments tending to drop off. Fertility and viability of males are good, but females are somewhat weak.

Fused, f (antennae and tarsi)

A single male was found by C. H. BOSTIAN in February, 1929, with antennal segments fused together and tarsal segments lacking (plate 2, figures 52–55 and plate 3, figures 100–102). The wing of fused has a very characteristic and constant indentation at the apex (plate 1, figure 2). Fused appears to be of good viability and fertility in the male. The female cannot reproduce on account of inability to feed from caterpillars. On February 8, 1931, there was found by P. W. WHITING a second fused mutant occurring in a fraternity (2004.5d) segregating males as follows: type 18, semilong 7, cantaloup 15, semilong cantaloup 23, semilong cantaloup fused 1. The mother was from cantaloup female by semilong male. Daughters of this mutant crossed with males of fused stock produced fused daughters as well as fused sons, showing that the mutated locus was identical with that of the previous mutation. In February, 1931, there was also found by L. H. BENKERT a third fused mutation. An orange reduced female crossed with a spread male produced daughters which were isolated for linkage tests. One of these segregated males (A20.05 LHB) as follows: type 4, orange 7, reduced 3, orange reduced 2, fused 4, orange fused 5, orange reduced fused 4. These two recurrences of fused in the same month are noteworthy. No fused was being bred at the time except for some stock that was otherwise type. The two recurrences were moreover entirely independent.

Long, l (antennae and wings)

In June, 1929, there were found by P. W. WHITING a number of males with antennal segments elongated and distal portion of wings shortened and curved ventrally (plate 1, figure 12, plate 2, figures 47–48). Segments of the legs are somewhat longer and thinner (plate 3, figures 78–80) than in type (figures 75–77). A female from ivory defective stock (number 17) had been crossed with an orange miniature male. An orange daughter pro-

duced 50 of the mutants to 46 non-long. Long males from vial a were crossed back to the heterozygous virgin mother and thus long females were obtained, 10 long to 3 non-long. Long had evidently been running in stock (number 17) as two or three long males were found dead early in the year in this stock. By lucky chance a female heterozygous for the mutant factor had been selected for the mating above mentioned, and a heterozygous daughter produced the 50 mutants. Males of long are of good viability and fertility but females are rather weak.

Semilong, s_l (antennae and wings)

A male of type stock (number 11) was treated June 30, 1930 (filter, ½ mm aluminum; peak voltage, 76 K. V.; milliamperes, 8; distance, 15 cm; duration, 8 minutes; dosage, 2280 R units) by R. ELIZABETH CHALMERS and mated to three females (number 11) (July 7, 10, 17). One of the daughters from the last mating produced 6 type males and 6 mutant males which have been called semilong because of similarity to long. Semilong males are of good fertility and viability. Females are also viable and fertile but rather weak. Antennal segments (plate 2, figures 45–46) are lengthened but are not as attenuated as are those of long (figures 47–48). Leg segments are also somewhat lengthened but heavier than corresponding segments of long. Distinction of semilong as of long from type is clear in antennae and to a great extent in legs, but overlapping between long and semilong apparently takes place especially in legs. The wing of semilong (plate 1, figure 1) is to be compared with that of long (figure 12) on the one hand and with that of fused (figure 2) on the other. It is not, as in long, distally curved, but is quite flat. Distal shortening, however, takes place as is evidenced by the form of the outer margin and especially by the abbreviation of the first radial cell. A slight indentation is present at the apex of the wing, not however as pronounced as in fused. It was at first thought that semilong might be allelomorphic to long, but this proves not to be the case.

Confluent, c_f (wing veins and antennal segments)

On August 2, 1930, ANNA R. WHITING found (226.3c) a male with shortened wings showing fusion between first and third branches of radius at tip (plate 1, figure 25). The mother was a control in an experiment with X-rays. There were 52 male sibs plus the mutant in vial c and 195 male sibs in the entire fraternity. Figure 24 (plate 1) represents an approximation to type in form of wing. There is more or less fusion and deficiency of segments distally in the antennae (plate 2, figures 49–50). Degree of abnormality in wings is correlated with that of antennae. At slightly in-

creased temperature many confluent die in cocoons and at lower temperature confluent overlaps with type.

Truncate, t_r (wing veins and antennal segments)

On August 7, 1930, C. H. BOSTIAN found (260.4b ARW) a male with primary wings much shortened distally (plate 1, figure 5). The mother had been rayed (filter, ½ mm aluminum; peak voltage, 60 K. V.; milliamperes, 5; distance, 15 cm; duration, 32 minutes; dosage, 2600 R units) as a three or four day larva on July 15. There were 71 male sibs. Truncate is very irregular in appearance apparently having a considerable overlap into type. Terminal antennal segments are very likely to be more or less fused and wings are often but slightly affected. The variation suggests confluent in certain respects. In combination with reduced it is more easily recognized.

Unexpanded wings

In August, 1930, NEITA C. BOSTIAN found in vial b from a type stock female (number 1), 12 type males and three with unexpanded wings (plate 1, figure 4). About 15 brothers were in vial a, all normal. Wasps with this mutant trait were bred for several generations, but were highly inviable, the majority dying in cocoons. Females were obtained but were inviable and the stock was discarded.

Vestigial, v (wings)

On August 4, 1930, NEITA C. BOSTIAN found (72.2d NCB) a culture vial containing wasps with vestigial wings (plate 4, figures 125–126). The mother was virgin producing males segregating other traits in a test for linkage. In vials a, b, and c there were found 50 non-vestigial. In vial d there occurred 12 vestigial and 12 non-vestigial. The seven wasps in vial e were non-vestigial. Vestigial is semi-lethal, many wasps dying before eclosion. Six of the mutants were dug out of cocoons. No other dead occurred except those in vial d. Hence the mutants were all produced when the mother was approximately 14–17 days old. Part, only, of the ovary was involved. Vestigial is of good fertility in the males that eclose, and many of them live a normal length of time.

Tapering, t_a (antennae)

On May 2, 1930, LYSBETH H. BENKERT found, in pedigree culture (A97.4 LHB) from wild stock (Minnesota), 9 males with tapering antennae. There were 5 normal male sibs and 14 sisters. The mother was, therefore, probably heterozygous. Tapering has good viability and fertility in both sexes. Antennae are very deficient with much fusion and irregularity

of segments distally (plate 2, figures 56–65). Tapering does not overlap with type but reverse mutations have occurred (see Discussion).

Spread, s_p (wings)

On May 18, 1930, MAGNHILD M. TORVIK found (134.1 MMT) from a male fraternity of orange stock (number 3), eight males with wings spread out to the sides. Further examination showed that these males had light areas on the mesopleura in the position of insertion of the directive wing muscles (plate 4, figures 128–131). Eight brothers occurred in the same fraternity. The mother, isolated from stock, was evidently heterozygous for the factor. Thus far it has been impossible to obtain offspring from female spread. Males are of normal fertility. The wings are themselves normal and the defect is probably muscular. The trait grades into type.

Broad, b_r (thorax)

In August, 1930, L. H. BENKERT found a number of males with thorax abnormally broadened (plate 4, figures 122–124). There is more or less tendency toward reduplication in the wings as illustrated in the figures. The mutant type was first noticed in about 50 percent of the sons of two different sisters. The character is somewhat irregular and it cannot be stated with certainty that this represents the first appearance of the mutation. Broad may be called an irregular recessive but about 50 percent of the heterozygous females show the trait to a slight extent. Broad has good fertility and viability.

Notch, N_0 (wings)

A female with notched wings was found by L. H. BENKERT, April, 1930, from a cross of stock number 3 female by tapering male. There were 16 type sisters 3 of which produced notch males. Notch does not breed true, and it is very likely that there are more factors than one involved. In many cases the trait acts like a dominant lethal. There is much irregularity and asymmetry in expression (plate 1, figures 6–11).

Shot, s_h (wing veins)

In August, 1930, L. H. BENKERT found a number of wasps with irregular venation. They were the offspring of different parents that had been subjected to high temperature accidentally. There is great irregularity in expression of the character (plate 1, figures 32–42). Fertility and viability are good.

Kidney, k (eyes)

On August 18, 1930, ANNA R. WHITING found (276.6b) a male with compound eyes lacking with the exception of a very minute one on the right

side. Ocelli were of approximately normal size. The mother had been rayed (filter, ½ mm aluminum; peak voltage, 60 K. V.; milliamperes, 5; distance, 15 cm; duration, 32 minutes; dosage, 2600 R units) as a five day larva on July 22. There were 18 male sibs in vial a and 18 in vial b including the mutant. The mutant was reared under standard condition, 30°C, but the F_2 generation was bred at lower and variable temperatures. Eyes of mutant type in F_2 were elongated dorso-ventrally, some of them kidney shaped. Kidney is highly lethal at 30°C, many wasps dying as eyeless pupae. At lower temperatures, 25°C, for example, it is of excellent viability and fertility in both sexes. No lethal pupae were noted among the sibs of the mutant. It is therefore unlikely that the single mutant had more than one or two lethal kidney sibs at most.

Small head

A mated female from mixed stock was treated April, 1927 (filter, ⅓ mm aluminum; peak voltage, 40 K. V.; milliamperes, 25; distance, 15 cm; duration, 30 minutes), by P. W. WHITING (WHITING 1929, p. 273). She produced type males 7, type females 3. Her daughters were tested. Two produced only type. The third produced type females 28, type males 19, and 12 males with very small heads. Test of 11 of the sisters of the mutants showed 4 producing only type, 3 giving type and small head males, and 4 giving type and small head in both sexes, having evidently mated with their small head brothers. Small head was much more distinct in the homozygous females but showed considerable overlapping in the males. It was of good fertility and viability but was lost on account of interruption by other work.

DISCUSSION

Lethals

As is now well known from the extensive work on Drosophila, mutation rate can best be measured by the occurrence of lethals whose frequency is many times that of visibles. No other form has yet been sufficiently exploited genetically to make a study of rate of lethal production practicable. Habrobracon thus far is no exception to this rule. Sex ratio furnishing an excellent index of lethals in Drosophila is subject in Habrobracon to so many diverse influences (temperature, quantity of sperm available, genetic stock of male and of female and particular combination involved) that only in extreme cases can it be utilized as evidence of lethals. Balanced lethals or several independently segregating lethals might be expected to so reduce number of males as to be detected, but, on account of the hap-

loid nature of the males, either of these conditions would be rapidly eliminated. A lethal may likewise be detected by linkage with a visible. Abundant evidence of lethals exists in Habrobracon, but for the most part these have not been analyzed genetically.

After certain preliminary tests it has been decided that, for the present at least, it is much better economy to confine attention to the "best" visible mutant types and to seek to determine by means of these what may be regarded as "normal" genetic relationships. Visibles, when obtained, are therefore bred to type stock and no attempt is made to study irregularities that may be due to raying. A genetic map must first be made.

Elimination of lethals must be very rapid in Hymenoptera and in other forms with haploid males. This does not of course presuppose that the germ-plasm of these types is any more stable, any less mutable. The more rapidly genetic degeneration is brought to the surface the more rapidly is it eliminated.

Mutation rate in pure and mixed stock and in X-rayed material

Mutation in the locus orange associated with heterozygous parentage will be deferred for consideration under Recurrences and Reversions, treated below.

Considering first the untreated material we find 6 mutations in the unmixed Lancaster material, descended from a female taken in Lancaster, Pennsylvania, 1919. Of these mutations, 2 are to orange, 1 to light ocelli, 1 to lethal pupae, 1 to spread, and 1 to unexpanded. Possibly 300,000 wasps of this material have been passed under observation, most of them males. One mutation, tapering, was found in pure Minnesota stock, besides shot which occurred after peculiar conditions of high temperature. The amount of pure Minnesota material examined up to the present time is small relative to the Lancaster, but, on account of the type of work being done with it, it has been very carefully studied.

By far the great majority of mutants have been found in material of mixed origin, from recent crosses of different lines. These mutations are: 2 to ivory, 3 to fused, and 1 to each of the following: dahlia, wrinkled, reduced, long, confluent, vestigial, broad, notch, constricted, maroon. It is impossible to say how many wasps have been observed from mixed material but the number is many times that of Lancaster and Minnesota combined. To the list of pure stocks should be added a relatively small group of inbred material from Iowa City (number 11), about 20,000 of which have been studied, most of them very carefully, but no mutations occurred among these. The facts indicate that no marked difference exists in mutation rate between pure bred and mixed material.

X-ray mutations may be classified as follows according to the type of material treated: from Lancaster material, cantaloup; from Iowa City stock (number 11), beaded, twisted, narrow, wavy, semilong; from mixed material, small head, miniature, yellow, kidney, stumpy, truncate.

These mutations, like those from untreated material, are roughly distributed in relative frequency according to the stocks used in the experiments.

Increase in mutation rate by X-ray treatment is obviously very great as the following considerations indicate.

1. The X-ray experiments involve small numbers relative to the numbers which have been and are being observed in the extensive genetic tests on linkage, male biparentalism, mosaicism, etc.

2. Only certain counts in the X-ray experiments are such as can be expected to disclose mutations arising at time of raying. Other counts actually serve as controls and since a special point has been made of this fact, these "controls" have been as closely studied as the "experimental." The "controls" in the experiments are, moreover, more numerous than the "experimental."

3. Six of the X-ray mutant types above mentioned, namely, small head, twisted, beaded, semilong, wavy, narrow, and miniature, occurred in such numbers (50 percent of the males in a fraternity) that they would have attracted attention in any count in the regular genetic work. This means that every fraternity of wasps counted serves as an additional control to these experiments. Among many thousands of fraternities of untreated material only ten mutations have been found with several mutants appearing in the fraternity. Among about 800 fraternities from treated parents there occurred the six mutants.

4. Four X-ray mutants, cantaloup, kidney, stumpy, and truncate, occurring as single individuals, are so striking that they would not have been missed had they occurred among the hundreds of thousands of individuals in non-rayed material. Only 9 mutations (reduced, 2 orange, ivory, light, dahlia, 2 fused, confluent) have been picked up as single individuals in non-rayed material, 2 of these (light and dahlia) being less conspicuous than the 4 X-ray types mentioned.

Stage at which mutation occurs

Mutants appearing as single males are: orange (2 occurrences in type stock besides those complicated by parental heterozygosis considered below), reduced, fused (2 occurrences, the second in a fraternity of 64), confluent (in fraternity of 196; since confluent overlaps with normal there may

have been confluent sibs unnoticed), cantaloup (in fraternity of 6, the mother X-rayed as adult), kidney (in fraternity of 36, the mother X-rayed as five day larva), truncate (in fraternity of 72, the mother X-rayed as three or four day larva; since truncate overlaps with normal there may have been truncate sibs unnoticed), stumpy (in fraternity of 187, the mother X-rayed as one or two day larva). In these 10 cases it is possible that the mutation occurred in but 1 ovocyte. If the mutation occurred at time of raying we would expect, in the case of cantaloup, but one well developed egg to be involved. Ovogonial stages would be involved in the cases of truncate and stumpy and even of kidney, but it is altogether possible that only one ovum might be descended from the mutant cell, as the sister cells might form adjoining follicular and nurse tissue.

Cases in which more than one mutant male appeared will now be reviewed. The first appearance of ivory showed 4 mutants in a fraternity including 29 individuals from orange bearing eggs. Distribution of sibs, moreover, gave evidence that the mother was not heterozygous but that the mutation involved only part of her ovary. In the case of unexpanded, data are not complete, but the occurrence of 3 mutants to 8 normal sibs in vial b and the fact that about 15 brothers were observed in vial a, all normal, indicate a situation comparable with that of ivory. The culture was not continued to vial c.

With reference to vestigial, conditions indicate that mutation took place before separation of the *Anlagen* of the two ovaries, but, nevertheless, after several ovogonial divisions. In other words the mutation involved a cross section of both ovaries or all four ovarioles. The 50 type of vials a, b, and c indicate that the ventral region was not involved. The 12 type to 12 vestigial of vial d indicate that the tissue functioning for 4 or 5 days was heterozygous. Each ovary in Habrobracon is made up of two elongate ovarioles. If tissue in only one ovariole were involved we would expect a ratio of 7 type to 1 mutant, while if tissue in both ovarioles of 1 ovary were involved we would expect 3 type to 1 mutant. Failure of mutants to appear among the 7 type of vial e indicates that non-mutant tissue occurred in dorsal end of ovary. Numbers are unfortunately small but the above suggested hypothesis is apparently the most plausible.

Only 2 X-rayed females gave rise to more than 1 mutant male. One of these, a larva treated when 4 days old, developed into an adult producing 2 yellow (base of antennae) males in a fraternity of 84. One of these occurred in vial b and 1 in vial c, so that about 10 days may have elapsed between raying and laying of mutant eggs. The other treated female was among a lot of eggs and larvae of miscellaneous ages. She produced 8 type

males and 19 beaded. The numbers, showing a non-significant departure in favor of mutants from the 1 to 1 expected from a heterozygote (lethal beaded pupae were counted), indicate that the whole functioning ovary consisted of mutated tissue. If mutation occurred at time of raying, the mother must have been in the early embryonic stage or else part of the ovarian tissue was non-functional. If the mutation was not caused by raying the gene may have been received from a parent, in which case the mother of the beaded mutants would be completely heterozygous.

In untreated material mutant males produced by mothers presumably heterozygous are wrinkled, lethal naked pupae, tapering, spread, broad, fused (LHB), and long. In the case of the first three named, evidence indicates that mutation occurred not earlier than gametogenesis of one of the maternal grandparents of the mutants. Spread, broad, and fused (LHB) may have been carried for some generations in the stock and previous finding of dead males of long proves this point for that mutation.

Two dominant mutations, constricted and notch, appearing first in females, probably occurred in gametogenesis of one of their parents.

Of mutations occurring in spermatogenesis 3 were first evidenced in single daughters. Light, dahlia, and ivory (EJW) of the orange locus each involved a mutation from the type allelomorph. Light with 34 type sisters and dahlia with 36 appeared each as a single compound with ivory. Ivory was recessive in the orange-ivory compound "mutant" female with 113 expected type sisters.

Five mutations appearing from heterozygous mothers may be assumed to have occurred in spermatogenesis at time of X-ray treatment. Miniature, twisted, and narrow were each from a single mother, whose father had been rayed 6 days previous, in each case, to the mating which produced those mothers. Semilong was likewise from a single heterozygous mother whose father had been rayed 17 days previous to the mating producing her. Wavy appeared from 4 different females among 11 tested daughters of a single female which had been mated to a male treated 24 days previously. No other mutants were produced by daughters from his previous matings so that only a small part of testis material was affected. Each of these 5 treated males had been mated a few times previous to the mating resulting in the mothers of the mutants. Hence the treatment may be assumed to have affected spermatogonial tissue.

One mutation, small head, appeared among the offspring of 1 of 3 tested daughters of a treated mated female. The mother of these mutants was evidently heterozygous. If the mutation took place at time of raying

it may have occurred either in the egg or in the sperm within the treated female.

Recurrences and reversions

With but two exceptions, both to fused, all recurrences have been in the locus for orange. One of these was from type to orange. Type has mutated once directly to ivory, while the original ivory mutation was from orange. Hundreds of thousands of wasps have been bred and recorded whose mothers were homozygous type, orange, or ivory, but no further recurrences have been reported.

The situation is very different when type and ivory stock are mixed. In this case mutations or apparent mutations to orange are relatively frequent. Black-eyed females heterozygous for ivory occasionally produce, in addition to the expected black and ivory sons, an orange-eyed mutant male. Breeding tests show these to be identical with orange, *o*. It is not known whether the ivory gene or its allelomorph has mutated in this case. Somewhat more frequently males appear with eyes mosaic for black and orange (WHITING 1928). Tests of these show that they breed as parental type would lead one to expect, namely, black or ivory, or mosaic black and ivory. They are apparent mosaic mutants but have never bred as orange. Their mosaicism is explained by egg binuclearity. Their mutation, real or apparent, is not explained, nor is that of the orange mutants from heterozygous, Oo^i, mothers.

With the exception of these apparent reversions from ivory to orange in the mosaic males and the possible reversions in the orange mutant sons of heterozygous, Oo^i, mothers, the only reversions thus far reported are in tapering. Tapering is clearly distinct from type, showing no overlaps. Five or six instances of type wasps appearing in tapering stock which had been carefully isolated (NCB, KAG, LHB) were at first attributed to contamination or to a mistake. Further work with pure stock gave an unquestionable instance of reversion. KATHRYN A. GILMORE bred from an isolated virgin female from tapering stock, 34 tapering males and 1 type male. This male bred to a tapering female produced type daughters. The reversion was therefore genetic and occurred in ovogenesis. The evidence indicates that tapering reverts not infrequently and shows the original mutation to tapering to be not an irreparable genic "loss."

Contrasted effects of different genes in corresponding structures

Let us now review the effects of the different mutant genes on the wings (see figure 1 and plate 1). Notch exerts its effect on the margin of either

primary or secondary; the notches may be terminal or lateral. Truncate and confluent affect the margin distally, tending to cause coalescence of R_1 and R_3 distally. Narrow cuts off irregular slices of both wings on costal and inner margins. Miniature and beaded reduce the size, straighten the costal margin and elongate the tip of the primary (showing in form of the first radial cell). Secondary is also somewhat shortened. Beaded, moreover, enlarges fourth radial cell. Long and semilong shorten the wing distally (showing in form of first radial cell) and long causes a distal ventral curvature while semilong causes a very characteristic but slight indentation at the apex. In fused this indentation is much more pronounced. Wavy produces transverse waves especially in region of first radial cell affecting veins R_1 and R_3 and general wing surface in adjoining region. A host of genes of greater or less potency cause breaks or disappearance of vein R_4. The most effective of these that has been isolated we have called d_{II95}. Instability of this particular vein may be regarded as a "natural" phenomenon while other venation differences here noted are "artificial" being unable to survive in nature. Shot causes irregular scattering of veins, especially in the region of the fourth radial cell. Reduced causes vein R_4 to be missing in the majority of specimens but its particular incidence is in the region of vein m-cu where there is an irregular scattering of veins with m-cu usually, though not always, lacking; there is considerable shortening of wings probably due to this extreme reduction of veins. Broad tends toward reduplication in wings or parts of wings. Short reduces size without affecting proportions. In certain types like broad and beaded the membranes of the wings may occasionally remain separate giving the wing an inflated appearance.

As regards antennae, long lengthens and attenuates segments without increasing number, while semilong has a somewhat similar effect but without such great attenuation. Miniature both shortens segments and reduces their number. Extreme reduction and fusion is to be noted in fused. Terminal deficiency frequently occurs in truncate and confluent and is extreme in tapering. In beaded and some of the other mutants more or less antennal deficiency is often noted, and in wavy there is frequently distal depigmentation and fragmentation.

The legs of the mutants show a great variety of modifications. In long there is a noticeable lengthening and attenuation frequently accompanied by bending in the weaker parts of the segments (this bending is not shown in the specimen drawn). Semilong has the legs more substantial but on the whole resembling long more than type. The small legs of miniature

are reduced proportionally or at least there is no marked disproportion in the parts. The marked enlargement in diameter of the femora and distal part of the tibiae of beaded is very striking. In fused the legs are approximately normal to tip of metatarsi to which the claws are apparently attached, the four tarsal segments being omitted. In stumpy there appear an extreme shortening and irregular crowding of tarsal segments, including the metatarsal. Claws are thus brought very close to the tips of the tibiae. Heterozygous females show a slight tendency in this direction, the tarsal segments being somewhat shortened and often irregularly set together. The prothoracic metatarsal spur, present in a large percentage, is a striking and unexpected phenomenon. Irregular bending and malformation of the legs as in twisted is to some extent also present in constricted but in the latter there is to be observed a constriction in the femora, especially noticeable in the metathoracic legs.

SUMMARY

1. Discovery and brief description of 36 mutant types in Habrobracon are given, 24 being reported for the first time. Three were taken wild while 33 occurred in stock. Twenty-seven are classed as recessives, 5 irregular or intermediate, 3 partially dominant and 1 dominant.

2. Color, size, and form of many parts of the body are affected and several of the mutant genes have multiple effects.

3. Three new mutations affecting eye color have occurred in stock. One of these, dahlia, is allelomorphic with orange, making quintuple allelomorphs in this series.

4. As regards orange locus, type (black) has mutated once to light, once to dahlia, once to ivory, and twice to orange; orange has mutated once to ivory; and orange has appeared several times among sons of type mothers heterozygous for ivory. None of these changes have been associated with X-ray treatment except for a few of the last named group.

5. Fused (antennae) has appeared three times by separate mutation. Tapering (antennae) has reverted to type a few times.

6. No striking increase in mutation rate is attributable to mixture of stocks.

7. Twelve mutations were associated with X-ray treatment. Considering relative numbers counted, this fact indicates a striking increase due to raying.

8. The allelomorphs to orange, light, and dahlia, and a recurrence to ivory were each first detected in a single female carrying a maternally derived allelomorph to the mutant gene. This fact and the appearance of

5 recessive mutant types among sons of daughters of treated males indicate that genic change took place in spermatogenesis. One of these recessives, wavy (wings), appeared among the sons of 4 daughters of a treated male indicating mutation in spermatogonium at time of treatment.

9. Ten mutations appeared as single males (besides several ivory to orange reversions) indicating mutation late in ovogenesis. In the case of 5 others it is likely that mutation occurred early in the female germ tract. At least 14 mutant types appeared as sons of heterozygous mothers.

ACKNOWLEDGMENTS

I wish here to express my indebtedness to the Committee on Effects of Radiation on Living Organisms of the NATIONAL RESEARCH COUNCIL from which I have received support for technical assistance and special apparatus. The work was also aided by two grants from the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE received in 1928 and 1929. The radiation experiments were begun in 1927 under a grant from the Committee for Research in Problems of Sex (NATIONAL RESEARCH COUNCIL). Results of some of the experiments conducted at that time are reported here. Facilities of the Zoological Laboratories of the UNIVERSITY OF PITTSBURGH have been freely used and the Zoological Laboratories of the UNIVERSITY OF CHICAGO were kindly made available during the past summer. During the summers of 1928 and 1929 the work was conducted at the MARINE BIOLOGICAL LABORATORY, Woods Hole, Massachusetts.

I particularly wish to commend the excellent spirit of unselfish cooperation and enthusiasm of the group of graduate students and others working under my direction. Their names appear in connection with the mutants they have found, but I owe an especial debt to NEITA CORRIHER BOSTIAN and to LYSBETH HAMILTON BENKERT for their tireless work in connection with many of the technical details. The drawings have, for the most part, been made by KATHRYN A. GILMORE who has been most painstaking and conscientious in their execution. Figures 32, 43, 44, 52–65, 67–71 have been made by L. H. BENKERT.

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A GRAPH FOR STANDARD ERRORS OF RATIOS

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The conventional technique for measuring the reliability of an observed ratio of one type within a population is comparing the ratio with its standard error. Each such reliability test, then, involves solving for the standard error, σ, in the equation

$$\sigma = \sqrt{p(1-p)/N}$$

where p is the ratio, and N the size of sample from which the ratio was obtained.

Frequent use of ratios, as in genetics and biometry, involves much laborious calculation, which may be shortened by the use of PEARSON'S (1924) or EMERSON'S tables. A graphic device for obtaining σ, where not too great accuracy is required, would have possibly an advantage over the former in carrying further the solution of the above equation, and over the latter, of including more values of p and N.

Such a device is here presented in one form of the nomogram or alignment chart as a suggestion that nomography may prove of some usefulness in genetics. The essential feature is the representation on three parallel lines of values for three related variables, so that any straight line, cutting all three, intersects them at values of the three variables which will satisfy the relationship between the variables represented by the nomogram.

BOOTHBY and SANDIFORD (1921) have used a group of nomograms for shortening greatly the time for obtaining human metabolic rate from gasometric data; FELDMAN and UMANSKI (1922) have presented a nomogram similar to one of the above for expressing the Dubois equation for surface area of the human body from its weight and height. L. J. HENDERSON (1928) has devised a more extensive, but similar, graph for representing simultaneous conditions of a number of components of human blood.

In figure 1 are included four independent nomograms for σ, each applicable to a certain range of p values and together extending from 0.5 percent to 99.5 percent. In each, the first scale covers a p range, the third, an N range; and the second, the corresponding σ range: thus scales 1, 11 and 12; 2, 9 and 10; 3, 7 and 8; and 4, 5 and 6 compose the four graphs.

In general, given a ratio and the size of the sample, these values can be located on matched p and N scales. A straight edge between the two points intersects the matched σ scale at the corresponding σ value.

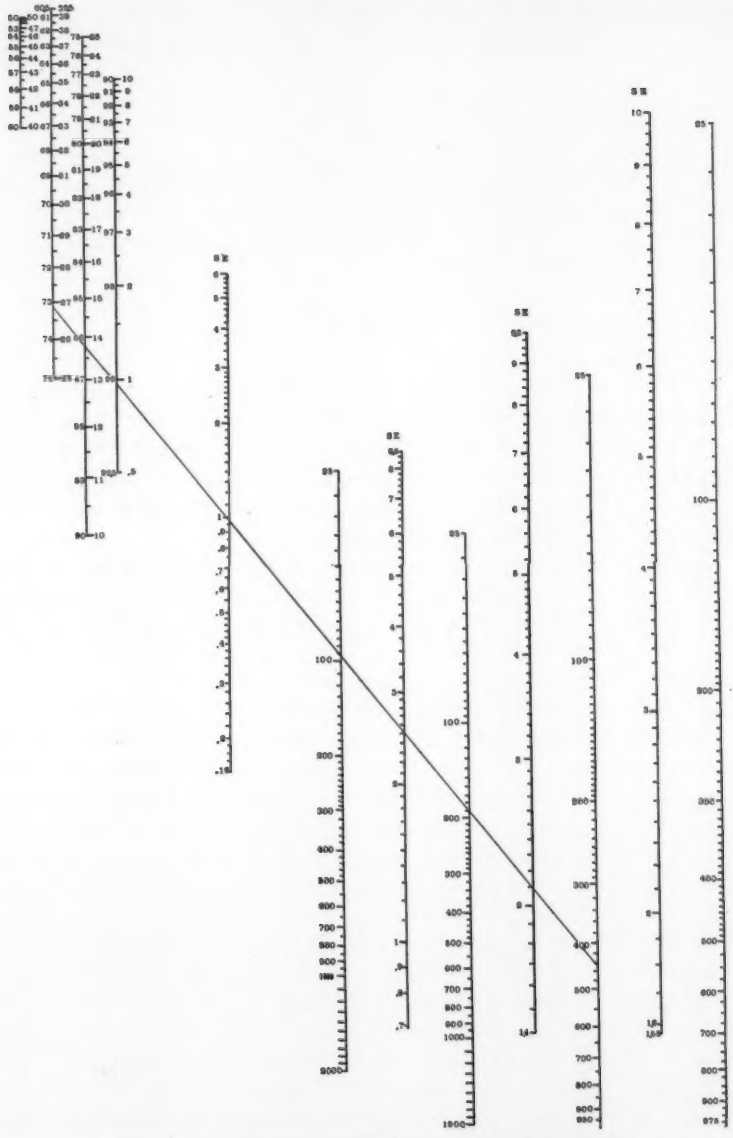

FIGURE 1.—Four nomograms for obtaining the standard error of a ratio.

Thus, with a ratio 26.9 percent determined from 451 individuals, one can locate these values on scales 2 and 10, respectively, of figure 1. A straight edge between the two points intersects scale 9 at 2.1 percent, the standard error of the given ratio with a sample of 451 individuals.

Careful readings do not differ from calculated values of σ by more than ± 1 in the second figure, which falls in either the first or second decimal place when σ is expressed, as here, in percent. With practice one can make the two coincide.

The three-line nomogram can be used for graphic solution of any equation in which the dependent variable, or its logarithm, can be expressed as the sum, or difference of some linear functions of the two independent variables, or the logarithms of these functions.

Here the standard error equation has been expressed as

$$\log \sigma = \tfrac{1}{2} \log p(1-p) - \tfrac{1}{2} \log N$$

The heights on the p scales are actually proportional to the values of $\frac{1}{2} \log p(1-p)$ for the p values shown, and, on the N scales, to $\frac{1}{2} \log N$ for the values indicated. The σ scales then represent the difference of the two expressions, which is $\log \sigma$.

The construction of a three-line nomogram is a fairly simple matter since, once lengths for any two scales have been decided upon, the length of the other, size of units of all three, and distances between scales are thereby determined and obtainable from simple relationships given by FELDMAN (1923) in a lucid discussion of nomography.

In table 1 are included the data from which figure 1 was made (on two joined sheets of Schleicher and Schuell paper Number 916). Starting points of scales are given in terms of distance, in inches, from the left and lower borders, respectively; distances from these starting points are expressed in inches. The figure can be reduced any number of times, n, by dividing all dimensions of table 1 by $\sqrt{n}$.

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TABLE 1

Data for construction of nomograms shown in figure 1, on a 22 inch×36 inch sheet. Starting points of scales are given as distances, in inches, from left and lower borders, respectively, of sheet. The graph may be reduced any number of times, n, by dividing all given dimensions by $\sqrt{n}$.

p_1		p_2		p_3		p_4	
0 POINT AT 0,32		1,24		2,19		3,21	
SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT
40.0	0.00	25.0	0.00	10.0	0.00	0.5	0.00
40.5	0.35	25.5	0.64	10.5	0.94	1.0	2.99
41.0	0.68	26.0	1.26	11.0	1.83	1.5	4.73
41.5	1.00	26.5	1.86	11.5	2.67	2.0	5.95
42.0	1.29	27.0	2.44	12.0	3.47	2.5	6.90
42.5	1.57	27.5	3.00	12.5	4.23	3.0	7.67
43.0	1.82	28.0	3.54	13.0	4.96	3.5	8.32
43.5	2.06	28.5	4.07	13.5	5.66	4.0	8.87
44.0	2.28	29.0	4.57	14.0	6.32	4.5	9.36
44.5	2.49	29.5	5.06	14.5	6.95	5.0	9.80
45.0	2.67	30.0	5.54	15.5	8.15	5.5	10.19
45.5	2.84	30.5	6.00	16.0	8.71	6.0	10.54
46.0	2.99	31.0	6.44	16.5	9.25	6.5	10.87
46.5	3.12	31.5	6.86	17.0	9.76	7.0	11.17
47.0	3.23	32.0	7.27	17.5	10.26	7.5	11.44
48.0	3.40	32.5	7.67	18.0	10.74	8.0	11.70
49.0	3.51	33.0	8.06	18.5	11.20	8.5	11.94
50.0	3.55	33.5	8.42	19.0	11.65	9.0	12.16
		34.0	8.78	19.5	12.08	9.5	12.38
		34.5	9.12	20.0	12.49	10.0	12.57
		35.0	9.45	20.5	12.89		
		35.5	9.74	21.0	13.28		
		36.0	10.07	21.5	13.65		
		36.5	10.36	22.0	14.01		
		37.0	10.63	22.5	14.36		
		37.5	10.90	23.0	14.70		
		38.0	11.16	23.5	15.02		
		38.5	11.40	24.0	15.34		
		39.0	11.63	24.5	15.64		
		39.5	11.85	25.0	15.94		

TABLE 1 (continued)

σ_1		N_1		σ_2		N_2	
6.5	11.48	10	12	20	3.32	14	19
SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT
0.157	0.00	25	0.00	0.69	0.00	25	0.00
0.160	0.18	30	0.79	0.70	0.12	30	0.79
0.170	0.34	35	1.46	0.80	1.09	35	1.46
0.180	0.59	40	2.04	0.90	1.92	40	2.04
0.190	0.83	45	2.55	1.00	2.70	45	2.55
0.200	1.05	50	3.01	1.10	3.40	50	3.01
0.220	1.46	55	3.42	1.20	4.02	55	3.42
0.240	1.84	60	3.80	1.30	4.60	60	3.80
0.260	2.19	65	4.15	1.40	5.14	65	4.15
0.280	2.51	70	4.47	1.50	5.64	70	4.47
0.300	2.81	75	4.77	1.60	6.11	75	4.77
0.320	3.09	80	5.05	1.70	6.54	80	5.05
0.340	3.35	85	5.31	1.80	6.96	85	5.31
0.360	3.60	90	5.56	1.90	7.35	90	5.56
0.380	3.84	95	5.80	2.00	7.72	95	5.80
0.400	4.06	100	6.02	2.10	8.07	100	6.02
0.420	4.27	110	6.43	2.20	8.41	110	6.43
0.440	4.47	120	6.81	2.30	8.73	120	6.81
0.460	4.67	130	7.16	2.40	9.04	130	7.16
0.480	4.85	140	7.48	2.50	9.34	140	7.48
0.500	5.03	150	7.78	2.60	9.62	150	7.78
0.550	5.44	160	8.06	2.70	9.90	160	8.06
0.600	5.82	170	8.32	2.80	10.16	170	8.32
0.650	6.17	180	8.57	2.90	10.41	180	8.57
0.700	6.49	190	8.81	3.00	10.66	190	8.81
0.750	6.79	200	9.03	3.20	11.12	200	9.03
0.800	7.07	210	9.24	3.40	11.56	210	9.24
0.850	7.33	220	9.44	3.60	11.97	220	9.44
0.900	7.58	230	9.64	3.80	12.37	230	9.64
0.950	7.82	240	9.82	4.00	12.74	240	9.82
1.000	8.04	250	10.00	4.20	13.09	250	10.00
1.100	8.45	260	10.17	4.40	13.43	260	10.17
1.200	8.83	270	10.33	4.60	13.76	270	10.33
1.300	9.18	280	10.49	4.80	14.06	280	10.49
1.400	9.50	290	10.64	5.00	14.35	290	10.64
1.500	9.80	300	10.79	5.20	14.64	300	10.79
1.600	10.08	320	11.07	5.40	14.91	320	11.07
1.700	10.34	340	11.33	5.60	15.17	340	11.33
1.800	10.59	360	11.58	5.80	15.43	360	11.58
1.900	10.83	380	11.82	6.00	15.67	380	11.82
2.000	11.05	400	12.04	6.20	15.91	400	12.04
2.100	11.26	420	12.25	6.40	16.14	420	12.25
2.200	11.46	440	12.45	6.60	16.36	440	12.45
2.300	11.65	460	12.65	6.80	16.58	460	12.65
2.400	11.84	480	12.83	7.00	16.79	480	12.83
2.500	12.02	500	13.01	7.20	16.99	500	13.01
2.600	12.19	550	13.42	7.40	17.19	550	13.42
2.700	12.35	600	13.80	7.60	17.38	600	13.80
2.800	12.51	650	14.14	7.80	17.57	650	14.14
2.900	12.66	700	14.47	8.00	17.75	700	14.47
3.000	12.81	750	14.77	8.20	17.93	750	14.77
3.200	13.09	800	15.05	8.40	18.11	800	15.05
3.400	13.35	850	15.31	8.60	18.28	850	15.31

TABLE 1 (continued)

σ_4		N_4		σ_3		N_3	
6.5	11.48	10	21	12	3.32	14	19
SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT
3.600	13.60	900	15.56			900	15.56
3.800	13.84	950	15.80			950	15.80
4.000	14.06	1000	16.02			1000	16.02
4.200	14.27	1100	16.43			1100	16.43
4.400	14.47	1200	16.81			1200	16.81
4.600	14.67	1300	17.16			1300	17.16
4.800	14.85	1400	17.48			1400	17.48
5.000	15.03	1500	17.78			1500	17.78
5.200	15.20	1600	18.06			1600	18.06
5.400	15.36	1700	18.32			1700	18.32
5.600	15.52	1800	18.57			1800	18.57
5.800	15.67	1900	18.81			1900	18.81
6.000	15.82	2000	19.03				

σ_2		N_2		σ_1		N_1	
16	3.09	18	24	20	3.07	22	32
SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT
1.4	0.00	25	0.00	1.58	0.00	25	0.00
1.5	0.78	30	1.19	1.6	0.38	30	1.56
1.6	1.52	35	2.19	1.7	1.34	35	2.92
1.7	2.22	40	3.06	1.8	2.24	40	4.08
1.8	2.87	45	3.83	1.9	3.09	45	5.10
1.9	3.49	50	4.52	2.0	3.90	50	6.02
2.0	4.08	55	5.14	2.1	4.67	55	6.85
2.1	4.64	60	5.70	2.2	5.41	60	7.60
2.2	5.17	65	6.22	2.3	6.11	65	8.30
2.3	5.68	70	6.70	2.4	6.78	70	8.94
2.4	6.16	75	7.16	2.5	7.43	75	9.54
2.5	6.63	80	7.57	2.6	8.05	80	10.01
2.6	7.08	85	7.97	2.7	8.64	85	10.63
2.7	7.51	90	8.34	2.8	9.22	90	11.13
2.8	7.93	95	8.70	2.9	9.77	95	11.59
2.9	8.33	100	9.03	3.0	10.31	100	12.04
3.0	8.72	105	9.35	3.1	10.82	105	12.46
3.1	9.09	110	9.65	3.2	11.32	110	12.87
3.2	9.46	115	9.94	3.3	11.81	115	13.25
3.3	9.81	120	10.22	3.4	12.28	120	13.62
3.4	10.15	125	10.48	3.5	12.74	125	13.98
3.5	10.48	130	10.74	3.6	13.18	130	14.32
3.6	10.80	135	10.98	3.7	13.62	135	14.65
3.7	11.12	140	11.22	3.8	14.04	140	14.96
3.8	11.42	145	11.45	3.9	14.45	145	15.27
3.9	11.72	150	11.67	4.0	14.85	150	15.56
4.0	12.01	155	11.88	4.1	15.24	155	15.85
4.1	12.29	160	12.09	4.2	15.62	160	16.12
4.2	12.57	165	12.29	4.3	15.99	165	16.39
4.3	12.84	170	12.49	4.4	16.35	170	16.65
4.4	13.10	175	12.68	4.5	16.71	175	16.90

TABLE 1 (continued)

σ_2		N_2		σ_1		N_1	
16	3.09	18	24	20	3.07	22	32
SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT	SCALE READING	DISTANCE ABOVE ZERO POINT	SCALE READING	DISTANCE BELOW ZERO POINT
4.5	13.36	180	12.86	4.6	17.06	180	17.15
4.6	13.61	185	13.04	4.7	17.40	185	17.38
4.7	13.85	190	13.21	4.8	17.73	190	17.62
4.8	14.10	195	13.38	4.9	18.05	195	17.84
4.9	14.33	200	13.55	5.0	18.37	200	18.06
5.0	14.56	210	13.86	5.1	18.69	210	18.49
5.2	15.01	220	14.17	5.2	18.99	220	18.89
5.4	15.44	230	14.46	5.3	19.29	230	19.28
5.6	15.86	240	14.73	5.4	19.59	240	19.64
5.8	16.26	250	15.00	5.5	19.88	250	20.00
6.0	16.65	260	15.26	5.6	20.16	260	20.34
6.2	17.02	270	15.50	5.7	20.44	270	20.67
6.4	17.39	280	15.74	5.8	20.72	280	20.98
6.6	17.74	290	15.97	5.9	20.99	290	21.29
6.8	18.08	300	16.18	6.0	21.25	300	21.58
7.0	18.41	320	16.61	6.2	21.77	310	21.87
7.2	18.74	340	17.00	6.4	22.27	320	22.14
7.4	19.05	360	17.37	6.6	22.76	330	22.41
7.6	19.35	380	17.73	6.8	23.23	340	22.67
7.8	19.65	400	18.06	7.0	23.69	350	22.92
8.0	19.94	420	18.38	7.2	24.13	360	23.17
8.2	20.22	440	18.68	7.4	24.56	370	23.40
8.4	20.50	460	18.97	7.6	24.98	380	23.64
8.6	20.77	480	19.25	7.8	25.40	390	23.86
8.8	21.03	500	19.52	8.0	25.80	400	24.08
9.0	21.29	550	20.14	8.2	26.18	410	24.30
9.2	21.54	600	20.70	8.4	26.57	420	24.51
9.4	21.79	650	21.22	8.6	26.94	430	24.71
9.6	22.03	700	21.71	8.8	27.30	440	24.91
9.8	22.26	750	22.16	9.0	27.66	450	25.11
		800	22.58	9.2	28.00	460	25.30
		850	22.97	9.4	28.34	470	25.48
		900	23.34	9.6	28.67	480	25.66
		950	23.70	9.8	29.00	490	25.84
				10.0	29.32	500	26.02
						525	26.44
						550	26.85
						575	27.23
						600	27.60
						625	27.96
						650	28.30
						675	28.63
						700	28.94
						725	29.25
						750	29.54
						775	29.83
						800	30.10
						825	30.37
						850	30.63
						875	30.88
						900	31.13
						925	31.36
						950	31.59
						975	31.82

A NEW TYPE OF MOTTLED-EYED DROSOPHILA DUE TO AN UNSTABLE TRANSLOCATION*

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INTRODUCTION

The appearance of mottled-eyed flies in cultures derived from X-rayed parents of *Drosophila melanogaster* is of rather frequent occurrence. During the past two and a half years the writer has found thirteen mottleds in which the locus of white has been involved. Twelve of these were females and one was a male. Eight of the females proved to be fertile, and breeding tests showed that each behaved as an "eversporting" type. In addition to these cases, in which the mosaicism affected the eyes, there was found another eversporting type in which the fly, heterozygous for gray and yellow, had yellow spots on the body.

All of the mottleds obtained by the writer were found in F_1 cultures of which the X-rayed parent (usually the male) had in the X chromosome the wild-type allelomorph for white, and the untreated parent (female) carried the mutant gene for this color in at least one of the X chromosomes.

ORIGIN OF THE MOTTLED STOCK

The new mottled herein described was found early in December, 1930, in an experiment in which treated *Theta* males had been X-rayed (3710 r units) and crossed to untreated females, each with one ClB chromosome and a second X chromosome carrying the mutant genes for yellow, white, lozenge, and miniature. The *Theta* X chromosome had yellow and scute at the left end, and a duplicated fragment from another X chromosome attached to its extreme right or fiber end. This fragment represents a de-

* Part of the cost of the accompanying illustrations is paid by the GALTON AND MENDEL MEMORIAL FUND.

leted X chromosome and carries the wild-type allelomorphs of yellow, scute, and broad. There was found in the F_1 culture a gray notched female, in which the right eye was normal red, but the left eye had an area of white facets covering the lower fourth of the eye (figure 1). It was at first supposed that the fly was a "fractional" that had resulted from a break in one of the strands of the two-strand stage of the treated X chromosome. Such flies are sometimes found in F_1 cultures derived from treated X sperms.

In order to test the position and extent of the suspected break, the notched female was mated to a X-ple male ($s_c\ e_c\ c_t\ v\ g\ f$). The cross yielded forty wild-type females, ten gray notched females, one scute notched female, six yellow white lozenge miniature males, and five males representing crossovers, but no *Theta* males. All of the notched females had eyes with slightly roughened areas that were echinus-like in character.

For the next generation, the notched females were crossed to various types of males, the one showing scute being mated to a yellow white crossveinless miniature male. The progeny of this scute female showed that a crossover had taken place between the loci of lozenge and miniature, so that the right half of the broken X chromosome contained miniature, and the *Theta* fragment was no longer present. This explains why this female differed from her other notched sisters, for the elimination of the *Theta* fragment by the crossover allowed scute to show.

The progeny of the scute notched female was as follows: eleven wild-type females, four yellow females, eleven yellow miniature females, four X-ple males, one scute echinus male, and two scute, echinus, cut, vermilion males (the last three are crossovers). The variable mosaic condition of the eyes and wings of the yellow and yellow miniature females showed that the case represented a new mottled.

EXPLANATION OF THE MOTTLING

The yellow flies from this strain have been used for various breeding tests, and the results obtained have furnished the basis for a suggested explanation of the "eversporting" character of the changes responsible for the mosaic condition of the eyes and wings. The facts secured from the different breeding tests demonstrate that the mosaicism is due to an ***unstable translocation***. Genetic tests show that the left end of the treated X chromosome had been broken off and had become reattached to the fourth chromosome. The translocated piece on the fourth has the genes for yellow and scute, and the wild-type allelomorphs for prune, white, facet and echinus, the break having taken place between the loci of echinus and ruby.

A female zygote, receiving the broken X and its translocated fragment, together with a complete X chromosome with the mutant genes for yellow and white, may develop into a fly that appears normal for yellow and red. But in case the translocated fragment becomes lost from any cell during somatogenesis, the descendants of this cell will produce, in combination with cells that are not deficient, somatic tissue that exhibits mosaicism—in this case, mottled eyes, or notched wings, or both, depending upon whether the descendant cells happen to enter the embryonic rudiments of these structures. A male zygote receiving the broken X chromosome and the translocated fragment will develop into a yellow scute male. Such males are not very viable and are entirely sterile. They never have mottled eyes because (apparently) the loss of the fragment during development results in the death of the individual.

Hyperploid males and females are also produced. The hyperploid female has two complete X chromosomes and the translocated fragment, and she may have mottled eyes (in case the fragment is lost) but never shows notched wings. The hyperploid male has one complete X chromosome and the fragment. Such males are never notched, but may have mottled eyes; in fact, they constitute the only class of males that have mottled eyes.

This, in brief, is the explanation that we would offer to account for the eversporting character of the mottling and notching found in this strain of flies.

THE BREEDING TESTS

General account

The mottled notched stock has been maintained by crossing, in each generation, mottled notched females to yellow white singed males. There is a twofold purpose in carrying the stock by this method; the use of the mutant white brings out the mottling, and the presence of singed facilitates the detection of hyperploid flies.

The diagram given in text figure 1 shows how the non-crossover classes are produced in each generation. The diagram gives the distribution, in maturation and fertilization, of the sex chromosomes and the fourth chromosomes only, for we are not concerned with the behavior of the other two pairs of chromosomes (II and III). The composition of a yellow mottled notched female is indicated at the top of the diagram. She has the broken X chromosome, a yellow white singed X, one normal fourth, and a fourth with the attached translocated fragment. The missing piece at the left end of the broken chromosome is represented in white outline, while the corresponding piece linked to the fourth is shown in solid color.

The fragment has the mutant genes for yellow and scute; otherwise it contains wild-type genes throughout the remainder of its length.

The yellow mottled notched female will produce four kinds of gametes, and if these are fertilized by yellow white singed X-bearing sperms, four classes of females may be expected (1–4 of diagram). The first class is the yellow mottled notched females which are like their mother. The characters of this class of females are very variable, for their expression will de-

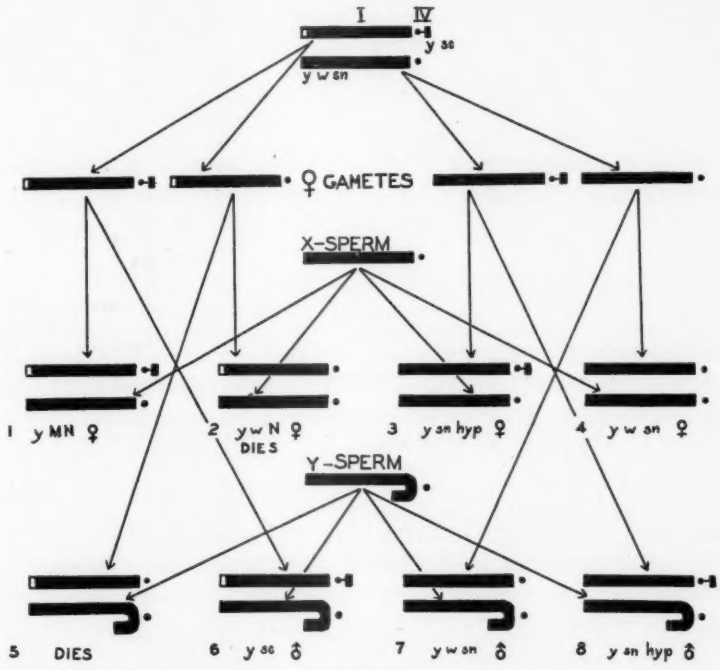


FIGURE 1.

pend on whether or not the translocated fragment becomes lost from the fourth during the course of development. If the fragment is not eliminated, the resulting female will appear as a normal yellow red-eyed fly. If the fragment happens to become lost during somatogenesis, the resulting female will show mottling, or notching, or both. The phenotypic expression of these two characteristics will therefore depend upon whether the deficient cells enter into their embryonic rudiments. One finds among this class of

LEGEND FOR PLATE 1

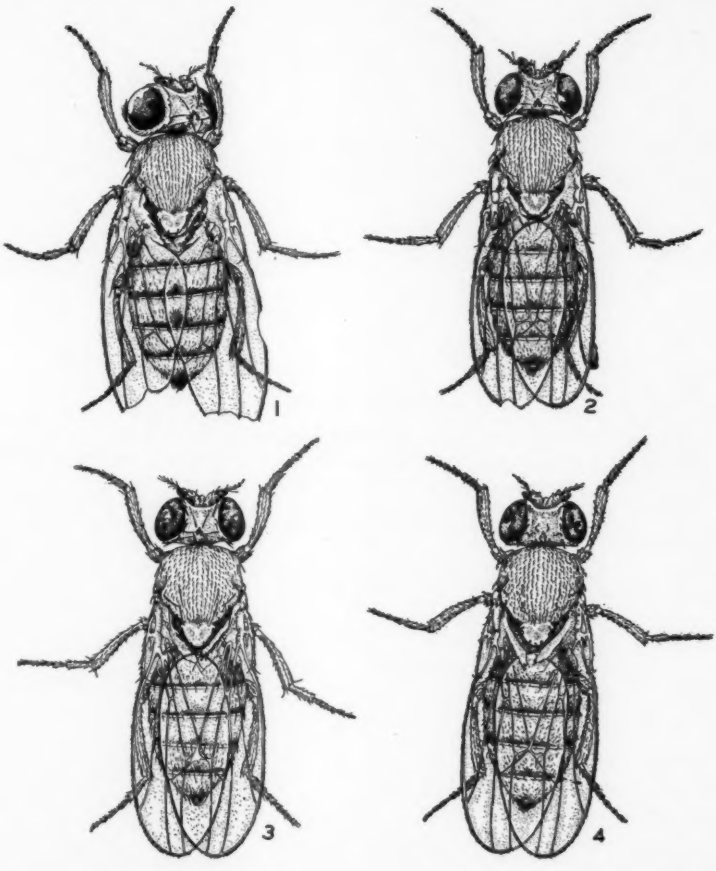
All of the drawings were made from the living flies by Miss SARAH L. BROOKS.

FIGURE 1.—Fly 231b. The original F_1 female from which the mottled notched strain was derived. The artist has represented the head as turned to the right, in order to reveal the single white area at the lower margin of the left eye.

FIGURE 2.—A female showing a small white area at the top of the right eye, and a slight notch in the left wing.

FIGURE 3.—Hyperploid mottled female. Note the abnormal posterior crossveins.

FIGURE 4.—Hyperploid mottled singed female.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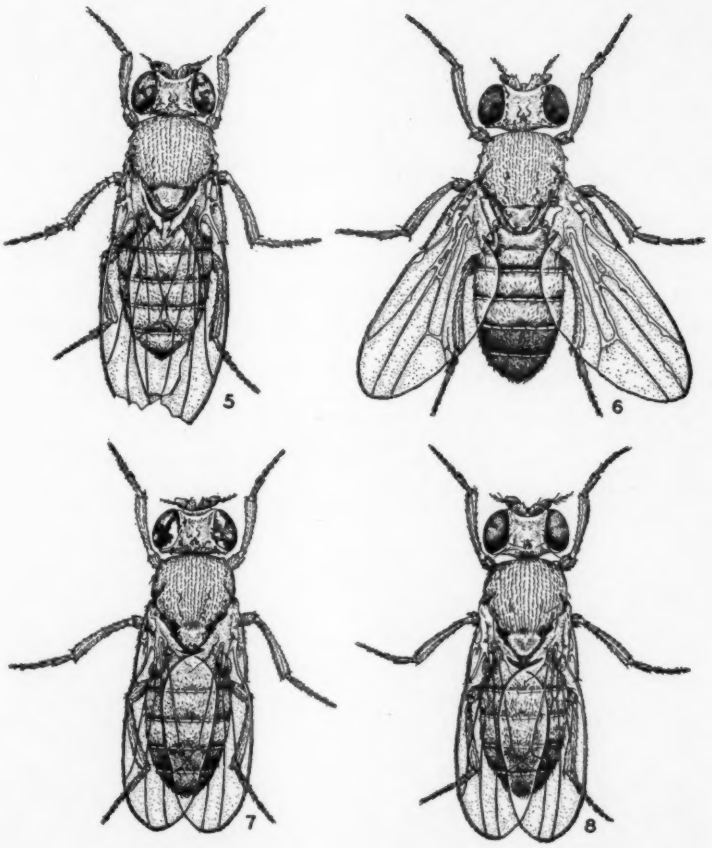
LEGEND FOR PLATE 2

FIGURE 5.—Non-hyperploid female, with mottled eyes and notched wings.

FIGURE 6.—A yellow singed hyperploid male, with abnormal wing veins and very rough eyes.

FIGURE 7.—A yellow singed hyperploid male, with mottled eyes.

FIGURE 8.—A yellow scute male. This male has the broken X chromosome and the translocated fragment on the fourth, which did not become eliminated during development.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LEGEND FOR PLATE 3

FIGURES 9–12.—Four wings showing various degrees of notching—from yellow notched females.

FIGURE 13.—Wing of a yellow hyperploid female.

FIGURE 14.—Wing of a yellow hyperploid female.

FIGURE 15.—Wing of a yellow singed hyperploid female.

FIGURE 16.—Wing of a yellow singed hyperploid male.

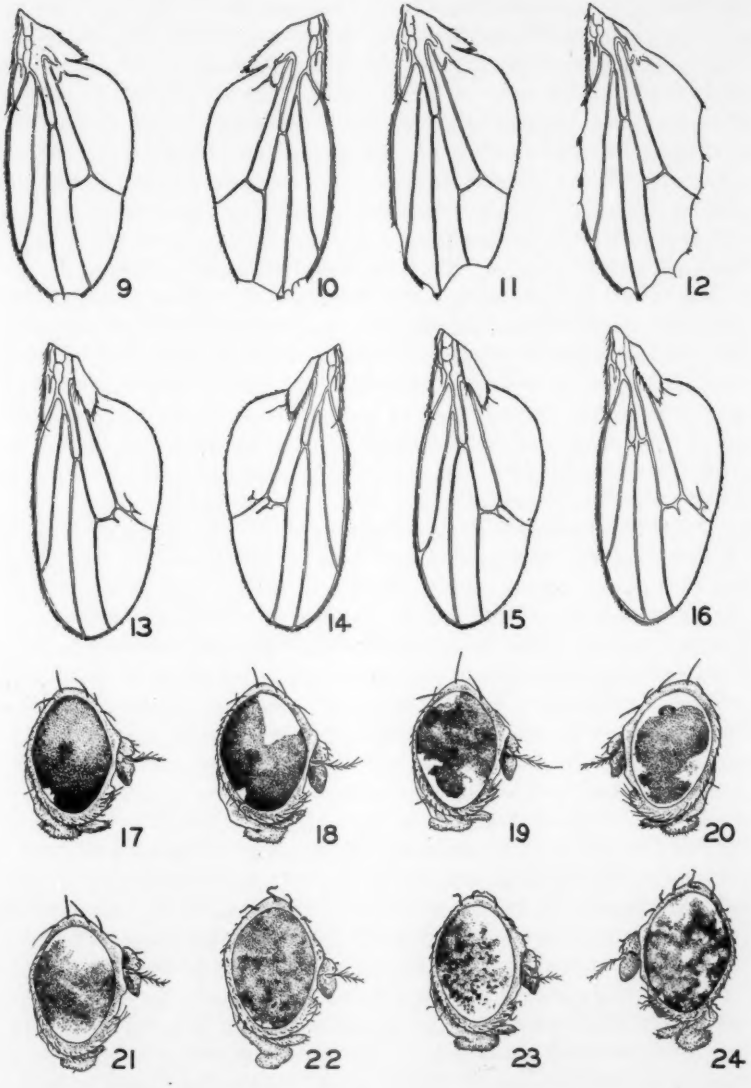
FIGURES 17–19.—Mottled eyes showing different degrees of mottling—from yellow mottled females.

FIGURE 20.—Eye of a yellow mottled notched female.

FIGURE 21.—Eye of a yellow mottled hyperploid female.

FIGURES 22–23.—Eyes of yellow mottled singed hyperploid females.

FIGURE 24.—Eye of a yellow mottled singed hyperploid male.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female individuals that show every degree of the mottled and notched conditions. The mottling may consist of a single small white patch on one eye (figures 2 and 17) or it may show as a comparatively large white area (figure 18), or as a mixture of red and white facets (figures 19 and 21). In some flies the eye may be nearly all white, with a few red flecks scattered over its surface. The notching is likewise very variable. In some cases there may be only a small nick in one wing (figures 2 and 9). Figures 10–12 illustrate wings from three females that show different degrees of notching. The mottling and notching are not only extremely variable, but they vary independently of each other, for it is not uncommon to find a fly with extensively mottled eyes but with non-notched wings, and *vice versa*.

The second expected class would be the yellow white notched females, but these are non-viable. However, they can be made to live by introducing into the genetic combination an X chromosome carrying an attached duplicated fragment. Thus if a yellow mottled notched female is crossed to a gray white *Theta* male, instead of to a yellow white singed male, gray white notched females are found in the next generation. The *Theta* fragment compensates for the loss at the left end of the broken X chromosome, and hence such zygotes are able to reach full development.

The yellow singed hyperploid females constitute the third class. As may be seen from the diagram, the hyperploid female has two yellow white singed X chromosomes, and the fragment attached to the fourth. They, too, show various degrees of mottling, due to the elimination of the translocated fragment. Since the hyperploid female has two unbroken X chromosomes, the elimination of the fragment will not result in producing notched wings; for it is well known that the condition of notch is due to a "deficiency" at the locus of facet, and consequently the loss of the fragment will not affect this locus in either of the two unbroken X chromosomes. The non-crossover hyperploid females can be recognized by the recessive character singed and frequently by the abnormal condition of the veins of the wings. In such females the posterior crossvein is usually branched, and in typical cases this vein shows a pointed-like projection extending posteriorly (figures 4 and 15). The branching may also involve the fifth longitudinal vein (figures 13 and 14). A few cases have been found in which the anterior crossvein extends across the sub-marginal cell (figure 16). The abnormal branching condition of the veins, like the mottling and notching in the first class of females, is also variable. It is not uncommon to find a female with one wing having normal veins and the other showing the typical hyperploid condition. In one culture yielding 84 yellow singed hyperploid females, it was found that in 43 cases only one wing had branched veins, while in the remaining 41 flies both wings showed this

condition. In some hyperploids (crossover classes) the wings may be normal. This variation in the expression of hyperploidy in the wing venation must be due to the same cause that underlies mottling, namely, to the loss of the fragment in the cells that form the embryonic rudiments of the wings.

The fourth or final class of females is represented by the yellow white singed females, which need no further comment.

If these same gametes are fertilized by Y-bearing sperms, four classes of males are possible (classes 5–8, bottom of diagram). The first possible class would be notched males, but since they must carry a deficient X chromosome, they would not be viable.

The yellow scute males constitute the second class (6). This type of male is not very viable, and, as numerous tests have shown, he is entirely sterile. The yellow scute male never has mottled eyes, because the loss of the fragment at any time would cause the death of the cell, and presumably of the zygote.

The regular yellow white singed males form the third class (7).

The fourth and final type of male expected is the yellow singed hyperploid male (8). This type of male is easily recognized, not only by the singed bristles, but also by his extremely rough eyes and highly abnormal wing veins. The veins are often swollen and branched. A typical case is seen in figure 6. Sometimes these males have mottled eyes (figure 7). The hyperploid males are not especially viable, but are more so than the yellow scute males. They are extremely infertile. About sixty of them have been tested for fertility and in only one culture did any larvae appear. None of these develop into an adult fly. They must occasionally be fertile, for PAINTER found division figures in the ovaries of a mottled-eyed female that showed the cells were homozygous for the translocated fragment, indicating that this female arose from an egg that had been fertilized by an X-bearing sperm having the attached fragment on its fourth chromosome. Hyperploid males can be produced that are not only quite viable, but are also distinctly fertile. This can be done by substituting the normal X chromosome for the yellow white singed X. In one experiment out of 196 males from one culture, 58 were wild-type hyperploids, that is, each had a wild-type X and the translocated fragment. A few of these males were mated to yellow females with attached X chromosomes, and the culture gave 27 yellow females, 24 yellow hyperploid females, 20 wild-type males, and 10 wild-type hyperploids. Evidently, the presence of the three mutant genes, yellow, white and singed, together with the fragment, tends to produce non-viability and sterility in the hyperploid male.

The elimination of the fragment from the cells of the hyperploid male has the same effect as in the hyperploid female, namely, it results in the formation of mottled eyes, and in the loss of the hyperploid type of wing venation. One very striking illustration of these effects was found in a hyperploid male in which the eyes were mottled. Each eye was white, with the exception of an area of about twenty-five red facets. It was noticed that each of these red patches had the characteristically roughened condition of the hyperploid eye, while over the white parts of the eyes the ommatidia were smooth and normally arranged. Furthermore, one wing of this male had normal veins, while the other wing showed the extreme plexus character of the typical hyperploid. The conditions found in this fly are exactly what one would expect to find when the extra fragment, that obviously causes the abnormal wings and eyes, has been eliminated from the cells of these structures. They should and do show normal conditions.

So far we have considered the non-crossover classes of flies only. Breeding tests show that while the amount of crossing over between the broken X chromosome and its yellow white singed partner is considerably reduced, yet it takes place with a frequency sufficient to produce several classes of crossovers. A single or double crossover between these two chromosomes will produce one X chromosome with the mutant genes for yellow and white, and another X chromosome (the broken element) with the mutant gene for singed. If the female gametes with these X chromosomes are fertilized, the following crossover classes will be produced: (1) yellow white females, (2) yellow white males, (3) yellow hyperploid females (sometimes mottled), which can usually be recognized by the abnormal wing venation (figure 3) and by breeding tests, (4) yellow hyperploid males, (5) yellow singed females, which may have mottled eyes and notched wings (figure 5), and (6) yellow scute singed males.

Tests with different types of females

Breeding tests have been carried out on five different types of females, namely, yellow, yellow mottled, yellow notched, yellow mottled notched, and yellow hyperploid females. Many individual matings were made, and the combined data are displayed in the accompanying table (table 1). In all the cases the females were crossed to yellow white singed males. The total number of offspring obtained from these tests was 6,416, and, as the table shows (first vertical column), these are grouped under twenty-one different classes. The classification is based on phenotypic differences. The main object of these breeding tests was to find out whether the different types of females would give offspring showing significant differences in the percentages of the several classes.

TABLE 1

CLASSES OF OFFSPRING	TYPES OF CROSSES					TOTALS
	YELLOW ♀ × *y w* s_n ♂	YELLOW MOTTLED ♀ × *y w* s_n ♂	YELLOW NOTCHED ♀ × *y w* s_n ♂	YELLOW MOTTLED NOTCHED ♀ × *y w* s_n ♂	YELLOW HYPERPLOID ♀♀ × *y w* s_n ♂	
y w s_n ♀ ♀	256	339	281	339	173	1388
y w s_n ♂ ♂	259	361	279	315	187	1401
y w ♀ ♀	11	41	10	48	248	358
y w ♂ ♂	16	34	10	46	187	293
y ♀ ♀	92	167	177	117	13	566
y M ♀ ♀	29	125	49	84	6	293
y N ♀ ♀	50	58	41	41	0	190
y M N ♀ ♀	95	42	67	109	0	313
y hyperploid ♀ ♀	12	43	19	6	108	188
y hyperploid *M* ♀ ♀	9	8	6	22	28	73
y s_n ♀ ♀	23	56	30	13	14	136
y s_n *M* ♀ ♀	14	52	12	18	1	97
y s_n *N* ♀ ♀	5	6	7	1	0	19
y s_n *MN* ♀ ♀	9	1	6	12	0	28
y s_n hyperploid ♀ ♀	92	28	98	66	44	328
y s_n hyperploid *M* ♀ ♀	114	99	116	152	112	593
y s_e ♂ ♂	5	16	19	7	0	47
y s_e s_n ♂ ♂	0	1	0	2	0	3
y s_n hyperploid ♂ ♂	17	40	19	4	9	89
y s_n hyperploid *M* ♂ ♂	2	5	1	2	0	10
y hyperploid ♂ ♂	0	2	0	1	0	3
Totals	1110	1524	1247	1405	1130	6416

The first four types (columns 2–5) may be considered together and compared, while the fifth type, the yellow hyperploid females, will be treated separately. The percentages of all of the yellow white offspring (lines 1–4) for the four types of females are 48.8, 50.8, 46.5, and 53.2, respectively (from left to right in the table). For the yellow red-eyed female offspring, the percentages run 8.3, 10.9, 14.2, and 8.3 (line 5). The yellow mottled, yellow notched, and yellow mottled female offspring gave percentages of 15.7, 14.8, 12.6, and 16.7 (lines 6–8). The yellow hyperploid classes show percentages of 1.9, 3.3, 2.0 and 2.0 (lines 9 and 10). The four classes of yellow singed females (lines 11–14) may be considered together, and the percentages of these are 4.6, 7.5, 4.4, and 3.1. Finally, the two yellow singed hyperploid classes have percentages of 18.5, 8.3, 17.1, and 15.1 (lines 15 and 16). These figures indicate that the percentages of offspring for any class are strikingly similar for each of the four types of mothers that were tested. A normal yellow red-eyed mother produces as many mottled and notched offspring as are produced, for example, by mottled notched

mothers. The slight differences in percentages are obviously due to chance variations.

The progeny of the yellow hyperploid mothers are given in the sixth vertical column of the table. The yellow hyperploid female had a yellow white singed X chromosome, a yellow white X chromosome (crossover X), and the translocated fragment. Such a female can be recognized by the hyperploid venation of her wings. All of the yellow white offspring will be non-hyperploids, while all of the red and mottled-eyed progeny should be hyperploids. It was found that the 795 white eyed offspring were non-hyperploids, but 34 of the 335 red and mottled eyed flies had normal wings, and it might, therefore, be concluded that they were also non-hyperploids. However, as suggested in the previous section, such flies are, in all probability, really hyperploids in which the fragment was eliminated from the cells forming their wings. From the nature of the chromosomal composition of the hyperploid mothers, notched-winged offspring would not be expected to appear, and, as an inspection of the table will show, none was found.

It is of interest to compare the number of red-eyed males among the offspring with the number of white-eyed males, for this will bring out the lack of viability of the former class. The total number of male offspring listed in the table is 1846, and of these 1694 were white-eyed, and only 152 were red or mottled eyed.

Tests for the length of the translocated fragment

It is a matter of importance to determine exactly what portion of the *Theta* X chromosome had been broken off and translocated to the fourth chromosome. This can be done by making suitable genetic tests. The results obtained in the first experiments demonstrated that the locus of white (wild-type allelomorph) was included in the translocated fragment. Furthermore, the appearance of notched wings indicated that the locus for facet was also included in the fragment. Tests have since been made for three loci lying to the right of that of white in the genetic map, and for two lying to the left of white.

The three loci lying to the right of white, and for which tests were made, were facet, echinus and ruby. It had been observed that a female having the broken X chromosome and its fragment, and another X chromosome with the mutant gene for facet, frequently had *exaggerated* (MOHR 1923) facet-like areas on her eyes. If the locus of facet is in the fragment, and the fragment is occasionally eliminated in somatogenesis, we should expect to obtain exactly this result. A much more convincing result may be obtained by the use of hyperploid females. These were produced by first crossing a mottled notched female to a facet male, and then backcrossing

the F_1 notched females to facet males. Some of the F_2 females will be hyperploids, that is, they will have two facet chromosomes and, in addition, the fragment on the fourth chromosome. All such females were found to have normal eyes, except two, and these had facet-like areas on their eyes, due to the occasional loss of the fragment. These results demonstrate that the fragment carries the wild-type gene for facet; for if this were not the case, then the hyperploid females, being homozygous for facet, would have had facet eyes.

The second locus tested for was that of echinus. In the first test made, the original notched females were mated to X-ple males (s_c, e_c, c_t v g f), and it was found that the F_1 notched females had eyes that were roughened, more exactly, had rough areas on their eyes. This result would suggest that the normal allelomorph for echinus lay in the fragment, but the evidence was not considered as conclusive, for the reason that females having notched wings often have rough-like eyes. It was therefore decided to make the "hyperploid test." The method followed for producing these was the same as the one used for facet. Mottled notched females were first crossed to X-ple males, and then the F_1 notched females were backcrossed to the same type of male. Eleven F_2 hyperploid females were secured, and, without a single exception, their eyes were non-echinus, thus showing that the wild-type gene for echinus was in the fragment.

In testing for the locus of ruby, the same general procedure was followed. The F_2 culture yielded eight hyperploid females and two hyperploid males, and all of these were ruby eyed. Evidently, the locus for ruby is not in the fragment, for if it were these hyperploid flies would have had red eyes.

The results obtained in the three tests outlined above showed that the break in the original *Theta* X chromosome (producing the fragment) had taken place at some point lying between the loci of echinus and ruby. However, there still remained the possibility that a second break had occurred, at some point lying to the left of the locus of white. That is to say, the fragment was not the product of a single break, but was the result of a deletion. The fact that yellow scute males were found in the cultures indicated that the extreme left end of the treated X chromosome was still present in the stock, but their appearance could be explained with equal plausibility, either by assuming that the extreme left end had become reattached to the remainder of the broken X, or by assuming that it was a part of the fragment. To settle this point, it was necessary to test points lying to the left of the locus for white.

The first locus test was that for prune. The hyperploid test was used, and the F_2 cultures gave seventeen females and one male that were hyper-

ploids. All of these flies had red eyes, demonstrating that the wild-type gene for prune was in the fragment.

The locus of scute was the second one lying to the left of white for which tests were made. The test for this locus is much more difficult than for any of the preceding ones, because of the fact that the *Theta* X chromosome, which was broken by the X-ray treatments, contained the mutant gene for scute. However, a satisfactory test can be devised by taking advantage of the facts developed by SEREBROVSKY and his associates relative to the different allelomorphs of scute. The allelomorph present at the left end of the *Theta* X chromosome is known to be s_c^1, and a "compound" of this and certain of the other allelomorphs of the scute series will give a fly having all of the bristles present, that is, phenotypically non-scute. I am indebted to Doctor I. J. AGOL for suggesting that his stock of s_c^{10} w^a would be a suitable allelomorph to use for making the test. The test was made by mating mottled notched females to $s_c^{10}w^a$ males, and then backcrossing the F_1 notched females. The F_2 flies of interest are the hyperploid females. A total of fifty-five such females was obtained, and without a single exception they were phenotypically normal or non-scute. Several of these females had mottled eyes (red and light apricot). These results demonstrate that s_c^1 is located in the translocated fragment, for if this were not so, the hyperploid females would have revealed s_c^{10}.

The facts developed from the several tests described in this section make it certain that the translocated fragment was produced by a single break, at some point lying between the loci for echinus and ruby. It cannot be less than 5.5 or greater than 7.5 map units in length (standard 1925).

Linkage tests for the translocation

It was suspected that the mottling was in some way due to a rearrangement of genes. MULLER (1930) has called attention to the fact that each case of mottleds so far found involves some type of rearrangement of genes: deletion, translocation, or inversion. Linkage tests were therefore carried out for each of the three autosomes. In testing for linkage to the second chromosome, yellow mottled notched females were mated to curly males. In the stock of males used the second chromosome has the dominant gene for curly and contains inversions that prevent crossing over. The F_1 notched curly females were then out-crossed to yellow white singed males. The F_2 generation gave eight yellow curly mottled females and seven yellow non-curly mottled females. Since both the curly and non-curly females show mottling, it is evident that the piece is not linked to the second chromosome.

In the test for linkage to the third chromosome, yellow mottled notched females were crossed to star curly translocation $C_{III \times 2}$ males. In this stock

the second and third chromosomes are tied together, and the star curly factors serve as markers, while C_{III} x 2 prevents crossing over. The F_1 notched curly females were out-crossed to yellow white singed males, and the F_2 generation yielded both star curly and non-star, non-curly, and mottled eyed females. This result showed that the fragment was not attached to the third chromosome.

The test for linkage to the fourth chromosome was made with the use of bent stock. The mutant gene for bent lies in the fourth chromosome. Yellow mottled notched females were crossed to bent males, and the F_1 notched females were then mated to yellow white bent males. The F_2 cultures gave 99 bent non-mottled females, and 110 yellow non-bent mottled-eyed females, some of which (54) had notched wings. A few of the F_2 yellow mottled females were backcrossed to yellow white bent males and the F_3 culture had fifteen yellow mottled females, six yellow mottled notched females, twelve yellow white bent females, and seven yellow white bent males. These results make it certain that the fragment is linked with the small fourth chromosome.

The cytological tests

While the linkage tests were in progress, my colleague, Doctor T. S. PAINTER, began a cytological study of this stock, and soon discovered in oogonial divisions that the fragment was attached to one of the fourth chromosomes. We have already made a brief report on our genetic and cytological findings (1931). I am grateful to Professor PAINTER for permission to give here, in advance of his final report, a brief statement of the cytology. Very clear figures of dividing oogonia of females heterozygous for the translocation show one fourth chromosome to be of normal size and the other to be about four times as large. The fragment is therefore about three times as large as a normal fourth. In a heterozygous female larva metaphase plates were found showing figures similar to those seen in oogonial divisions, and near-by other cells show normal fourths. This demonstrates that the translocation may become lost during somatogenesis.

DISCUSSION AND SUMMARY

The genetic and cytological evidence presented in the main body of the paper supports the conclusion that eversporting characters of the mottling and notching found in this strain of flies are due to an unstable translocation. By this is meant that the piece of the X chromosome attached to the fourth chromosome is occasionally eliminated during somatogenesis. Since the translocated piece contains, among others, the wild-type allelo-

morph of white, its elimination from any cells, heterozygous for red and white, will result in the production of mosaic tissue in the eye. In the same manner, the elimination of the fragment from cells entering the wing rudiments results in giving rise to notched wings, by virtue of the loss of the locus of facet. It is thus established that in this case the cause of notching is due to an actual loss of a piece of the chromosome, and not to an inactivation of genes, or to a line mutation.

At this point it might be well to emphasize that mottled and notch in the present strain of flies can be segregated into distinct stocks. As a matter of fact, the fertile hyperploid flies really constitute a mottled line, from which a stock can be established. The gray white notched females, derived from the cross with *Theta* white males, may in turn be employed for the purpose of establishing a stable notched stock. The writer has obtained from X-rayed material several cases in which the left end of the X chromosome has been broken off, thus producing notched females. By crossing such females to fertile hyperploid males from the present strain, it will be possible to produce a new mottled notched stock. These stocks are now being developed.

When and how is this translocated piece eliminated? To judge by the variation in size of the mottled and notched areas, as seen in different flies, the loss may take place during any cleavage or larval stage. In some flies the eyes may be nearly all white, and both wings may be notched, thus indicating that the elimination must have taken place at a very early cleavage stage. The other extreme is seen in flies having a white area composed of a single facet, or at most of a very few facets, and with wings entirely unaffected. The loss of the fragment in such cases must have occurred at a very late larval stage, for it has been found that the elimination of the locus of white through a breakage caused by X-rays produces white areas of this small size only when the treatment is given during late larval stages (PATTERSON 1929). It is not entirely safe, however, to conclude that, because the area is small, the elimination necessarily took place late in somatogenesis, for it is possible that the descendants of a cell, which lost the fragment early, have been distributed to many different body tissues where the detection of the elimination would not be possible. Nevertheless, in general a large deficient area would indicate an early elimination, and a small area a late loss.

Three possible methods of elimination may be mentioned and discussed; the first of these is by somatic non-disjunction. CLAUSEN (1930) has recently described a carmine-coral variegation in tobacco that he states is due to the occasional loss of an independent chromosomal fragment. He suggests that the loss may be due to somatic non-disjunction. In this

mottled-eyed fly no evidence supporting the theory of somatic non-disjunction has been found. If non-disjunction is the method of elimination, then it should be possible to demonstrate the fact by cytological studies, for, in that event, division figures showing a single fourth chromosome in the cell should be observed. Professor PAINTER'S observations have failed to reveal any such figures in the material.

It is possible to test the non-disjunction hypothesis by genetic methods. I am indebted to Doctor H. J. MULLER for suggesting the following experiment. The problem is to determine whether the fourth chromosome, to which the fragment is attached, is eliminated along with the piece. This can be done by using white eyeless males. White in the X chromosome is used to bring out the mottling, and the mutant gene for eyeless is in the fourth chromosome. Since the factor for eyeless greatly reduces the number of ommatidia, the relative size of white areas appearing in heterozygous females will show whether the fourth chromosome is lost at the same time elimination of the fragment occurs. If the fourth is not eliminated, then the white area would have the normal number of ommatidia; but if the fourth is also eliminated, then the white area should be reduced in size, for the recessive factor for eyeless in the other fourth chromosome would be able to express itself. The test was made by crossing mottled notched females to yellow white eyeless males. All of the white areas found on the eyes of the F_1 females were found to be of normal size, showing that the fourth chromosome, with the wild-type gene for eyeless, had not been eliminated. The results obtained from this test support the cytological evidence to the effect that non-disjunction is not the method of elimination.

A second possible method of elimination is one suggested and discussed by MULLER (1930). Concerning this method, he makes the following statement (MULLER 1930, pp. 327–328): "If, for example, it be postulated that the point of breakage and reattachment is a 'weak spot,' where the chromosome is liable to break again, with the resultant loss from some descendant cells of the section distal (with reference to the spindle-fibre attachment point) to the breakage, there arises the objection that the light or white areas in mottled males are nevertheless viable, although both theory and empirical findings point to the death of cells lacking such a portion of their only X chromosome." This criticism is clearly valid for mottled-eyed non-hyperploid males, but does not apply to mottled males that are hyperploids. The only type of mottled males found in our case is hyperploids, and consequently this second suggested method may account for the mottled females and males.

A third possible method of elimination is one suggested by the writer some time ago to account for the occasional loss of an attached fragment in another stock. It is, in brief, this, that when a somatic division takes place it is possible for the chromosome with the attached fragment to split in such a manner that one cell will receive a daughter chromosome bearing the undivided fragment, while the sister cell will receive the other daughter chromosome without the fragment. At the present time there is no way of determining which of these two possibilities, if either, is correct.

It would be of interest to determine whether any of the other mottleds that have been found can be explained on the basis of unstable translocations. Several such cases are now being investigated in our laboratories, but the work has not progressed sufficiently to enable us to give a definite answer. It would not be surprising to find that mottleds may be produced in various ways. In 1929 the writer found a mottled fly in which the mosaicism affected the body color only. The original female appeared in the F_1 culture in an experiment in which yellow white females had been crossed to X-rayed eosin singed males. The fly had yellow spots scattered over the body. Unfortunately, the stock was lost during the hot summer. In the meantime, breeding tests demonstrated that the yellow spotting, in flies heterozygous for gray and yellow, behaved as an eversporting type. Linkage tests showed that the extreme left end (with the wild-type allelomorph for yellow) had been broken off of the X chromosome and had become attached to the third chromosome. All of the evidence obtained at the time the stock was in hand indicated that this case was of the same general nature and could be explained in the same way as the present mottled case. Two other cases, apparently of the same nature, were found during this year. The translocated pieces in these include the loci for notched and miniature, respectively. Finally, attention may be called to the possibility that some of the numerous reported cases of eversporting variegated plants may be caused by unstable translocations. It is possible that the cases of endosperm chimeras in maize, recently reported by STADLER (1930), may be explained in this way.

SUMMARY

1. A new mottled notched stock of Drosophila is described and analyzed. The analysis shows that the eversporting character of the changes responsible for the mottling and notching is due to an unstable translocation.

2. The stock was developed from X-rayed material. The treated X chromosome had the left end broken off, and this fragment was reattached to the fourth chromosome, as shown by cytological and genetic evidence. The fragment carries the mutant genes for yellow and scute, and the tested

wild-type genes for prune, white, facet and echinus. The break therefore occurred between the loci of echinus and ruby, and in length the translocated fragment cannot be less than 5.5, or greater than 7.5 map units (standard 1925). Physically, the fragment is about three times the size of the small fourth chromosome.

3. The mottling of the eyes and the notching in the wings are both very variable, and vary independently of each other. The evidence shows that this is due to the occasional loss of the fragment during somatogenesis. Since the fragment has the normal allelomorph of white, its elimination will produce white areas (mottling) in a female having the mutant gene for white in her unbroken X chromosome. A similar loss will produce notched wings, by virtue of the fact that the fragment has the normal allelomorph for facet (the same locus as notch).

4. It is suggested that these changes will result whenever the descendant cells, of any cell that has lost the fragment during somatogenesis, happen to enter and form a part of the eye or wing.

5. Hyperploid flies appear in the culture. The hyperploid female has, in addition to the fragment on the fourth, two unbroken X chromosomes, and if each of these carries the mutant gene for white, she may have mottled eyes, but never shows notched wings. The hyperploid male has one unbroken X and the translocated piece. If his only X chromosome carries white, he too may have mottled eyes. A male that inherits the broken X and the fragment is yellow and scute, but he never has mottled eyes, because the loss of the fragment kills the cell, and presumably brings about the death of the zygote.

6. It has been possible to segregate the factors for mottling and notching into two distinct lines.

7. It is suggested that other cases involving eversporting mosaics may be due to unstable translocations.

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GENETICAL AND CYTOLOGICAL PROOF OF SOMATIC ELIMINATION OF THE FOURTH CHROMOSOME IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

The general importance of the genetical work in *Drosophila melanogaster* has to a considerable extent been due to the fact that it has in this material been possible, through detailed experimental analysis of exceptions to normal inheritance, to predict atypical cytological findings, predictions which were found to hold true when the cytological examination was carried out.

Since BRIDGES (1916) by this combined genetical and cytological method delivered the first conclusive proof of the chromosome theory of heredity, a number of cases have been described in which irregularities in the distribution of whole chromosomes during the maturation of the germ cells have led to chromosome aberrations, the nature of which has been cleared up by genetical methods and afterwards verified by cytological examination.

Aberrations from the standard chromosome equipment of the species may, however, also arise through irregularities in the chromosome distribution in somatic cell divisions, giving rise to different types of somatic mosaics.

For a survey of the various types of mosaics that are explainable as due to somatic elimination of one or more chromosomes see a recent paper of L. V. MORGAN (1929). Here only the following should be stated:

By far the most common type of somatic mosaics are the sex mosaics (gynandromorphs). As shown by MORGAN and BRIDGES (1919) the large majority of these gynandromorphs are due to somatic elimination in one of the early cleavage divisions of one member of the X chromosome pair. The individual starts as a female, but when one X is eliminated a daughter cell with a single X chromosome is formed. All those cells which are derived from this daughter cell will be X O and male, while the rest of the individual will be X X and female. The result is accordingly a gynandromorph consisting of one female and one male part. In most cases these gynandromorphs are more or less bilateral, but not infrequently the female part is larger than the male region.

Principally analogous to these sex mosaics are the so-called Diminished mosaics in which the body is divided into a normal part and a part showing the Dominant character complex Diminished. As shown by BRIDGES (1921) the Diminished character is not due to an ordinary gene, but to haploidy for the small round, so-called fourth chromosome. The Diminished mosaics have correspondingly been interpreted by BRIDGES as 2N in the normal and 2N–IV in the Diminished region, due to somatic elimination in one of the early cleavage cells of one member of the fourth chromosome pair (MORGAN, BRIDGES, and STURTEVANT 1925).

In *Drosophila melanogaster* BRIDGES has encountered about a dozen such mosaics, of which the majority were interpreted as IV IV in the normal and IV O in the Diminished part. But the possibility could not be excluded that some of them at any rate, were in reality due to a somatic mutation to a dominant Minute, since the Diminished character complex is strikingly like the character changes typical of the large group of dominant Minute mutations.

In his analysis of the claret mutant type of *Drosophila simulans*, STURTEVANT (1929) obtained no less than 171 Diminished mosaics of analogous type. This extraordinarily high number is due to the very peculiar action of the claret gene in homozygous condition. The gene causes irregularities in the chromosome distribution, not only during the maturation of the germ cells, but also in the early cleavage stages of the next generation, resulting in the production of a large number of non-disjunctional exceptions and gynandromorphs as well as of Diminished (haplo-IV) individuals (40 percent of all the offspring) and Diminished mosaics (4 percent of the total offspring). STURTEVANT obtained offspring from three Diminished mosaics, but these included no Diminished individuals.

There are also in *Drosophila melanogaster* a few cases in which particular genotypic changes increase the frequency of somatic elimination. Thus,

BRIDGES (1925) has described a sex-linked mutant Minute-n which causes a high percentage of elimination of the Minute-n X chromosome in somatic cells. And STERN (1927a) found that three different III-chromosome Minute mutations caused a high frequency of somatic elimination of particular sections of the third chromosome. In these cases the elimination occurs not in the cleavage cells but during the later larval developmental stages. Only a small part of the individual will accordingly be derived from the atypical cell, and the corresponding somatic alteration will be restricted to a limited region, frequently a mere spot in the surface area of the adult fly.

The genetic analysis of the mosaics resulting from different types of somatic elimination rests on the fact that each part of an individual develops according to its own genetical constitution (MORGAN and BRIDGES 1919).* Thus recessive genes present in the single X chromosome of a gynandromorph manifest themselves somatically in the male part. Haploidy for the fourth chromosome in one region of an individual produces the somatic change Diminished in this region, etc.

By taking advantage of this fact, and by using (or intentionally introducing) "marked" chromosomes containing different mutant genes it has been possible to carry out an analysis that is in most cases fully conclusive, in spite of the fact that the analysis primarily rests on the external inspection of the mosaic without crucial breeding test and cytological control.

As regards those gynandromorphs which are explained by simple single elimination, half of these can also be explained as derived from binucleated eggs. But there is here special genetical evidence in favor of the former explanation. Thus, gynandromorphs with apparently normal male abdomen are always sterile, like the X O males due to primary non-disjunction (MORGAN and BRIDGES 1919); the sex-linked bobbed character, which is suppressed in ordinary X Y males through the action of the normal allelomorph of bobbed present in the Y chromosome, manifests itself in the male region of gynandromorphs as it does in non-disjunctional X O males of bobbed stock (STERN 1927b); and in a large experiment which was planned in such a way that the chances of obtaining flies positively diagnostic for a binuclear origin were increased by the use of marked autosomes, not a single such fly was obtained, although the experiment produced 12 gynandromorphs that were explainable by simple elimination (L. V. MORGAN 1929).

* For certain exceptions to this rule see a recent paper of DOBZHANSKY (1931).

Cytological evidence in favor of the elimination explanation was obtained by PEARSON (1927) in gynandromorphs of the grasshopper Amblycorypha. The gonads contained both ovarian and testicular tissue, and the ovarian cells had two X chromosomes, while the spermatogonies had only one X.

The reasons why it has not, in mosaicism due to somatic elimination, been possible to deliver the complete documentation by combined breeding test and cytological demonstration are different:

On the one hand there is in Drosophila for technical reasons no chance of obtaining cell division figures from both regions of an adult mosaic, and mosaics are not detected in the larval or pupal stages; on the other hand the elimination of a chromosome only in particular cases leads to fertile chromosome combinations; even then the crucial breeding test can, of course, only be carried out if the gonads, or part of the gonads, happen to be included within the atypical and fertile part.

The mosaics which might be expected to offer the best chance of securing the combined genetical and cytological proof of somatic elimination are accordingly those which consist of one 2N and one 2N–1 region, and in which the elimination has occurred so early during the cleavage stages that part of the germinal tissue is derived from that cell which has lost one chromosome by elimination. In this case the atypical part, if fertile, will produce germ cells, half of which will be N–1. When these are fertilized by (or fertilize) normal N germ cells, the result will be 2N–1 individuals whose atypical chromosome equipment may be analyzed by appropriate genetical analysis, and of which there will be available a sufficient material for cytological examination.

The commonest type of somatic mosaics, namely, the ordinary gynandromorphs, do not fulfill the above requirements. In the Drosophila gynandromorphs both gonads are in most cases the same, either ovaries or testes. If the genital apparatus is female the gynandromorph, if fertile, breeds as an ordinary female. If the gonads and external genitalia are male, then the gynandromorph is completely sterile.

As regards those mosaics which might be expected to be produced by somatic elimination of the second or third chromosome, no such have ever been encountered, nor have haplo-II or haplo-III individuals been reported from any experiment. Obviously, the lack of one member of the second or third chromosome pair causes such gross changes that cells of this type are unable to develop.

There remains, however, one type of somatic mosaics which, under favorable conditions, may fulfill the requirements outlined above, namely,

the Diminished (haplo-IV) mosaics. If in a Diminished mosaic the elimination of one member of the fourth chromosome pair has occurred so early during the cleavage stages that part of the gonads are derived from the haplo–IV daughter cell, then there is the chance of obtaining offspring from this haplo-IV part. There is reason to assume that this part will be fertile, since we know from the Diminished individuals that the 2N–IV combination is a fertile combination.

Half of the germ cells from the haplo–IV region of such a mosaic will be functioning nullo-IV germ cells, and when these meet normal haploid germ cells, the result will be Diminished, haplo–IV individuals in which the lack of one member of the fourth chromosome pair may be demonstrated by genetical methods and by cytological examination as well.

It was the latter situation that was realized in the case to be described in detail below.

A preliminary account of this analysis was read before DET NORSKE VIDENSKAPSAKADEMI I OSLO (MOHR 1930).

THE OCCURRENCE AND DESCRIPTION OF THE "MINUTE" MOSAIC FEMALE

During the analysis of a new III-chromosome dominant mutant Vein (*V*), heterozygous females which in one member of the third chromosome pair carried roughoid (r_u, 0.0) and Vein, while the other member was wild-type, were mated singly to males homozygous for the following third chromosome recessives: roughoid, hairy (h, 26.5), thread (t_h, 42.2), scarlet (s_t, 44.0), curled (c_u, 50.0), stripe (s_r, 62.0), sooty (e^s, 70.7), and claret (c_a, 100.7). One of these cultures (C.4709, March 6, 1930) gave the following offspring: roughoid Vein 62, wild-type 55, roughoid 10 and Vein 7.

In the wild-type class a single individual was detected (March 12, 1930) which was a typical bilateral mosaic, consisting of a normal right half and a left half which had distinctly shorter and more slender bristles and hairs, typically like those present in the different Minute mutations. The left wing was in addition shorter and more bluntly rounded than the normal right wing (figure 1). The exceptional individual was in other respects a typical female; judging from the slightly swollen abdomen, there was reason to believe that she had already been fertilized.

In the head the border line separating the Minute and the wild-type region followed the median line strictly, both on the dorsal and on the ventral surface. This could easily be controlled by aid of the head bristles and larger hairs which were without exception Minute on the left and wild-type on the right side. The eyes were of about equal size, but the left

antenna and arista, as well as the left half of the distal surface of the proboscis, were smaller on the left than on the right side.

Also on the thorax the border line followed the median line strictly both on the dorsal and on the ventral side, all the bristles and larger hairs, including the sterno-pleurals, being Minute on the left and wild-type on

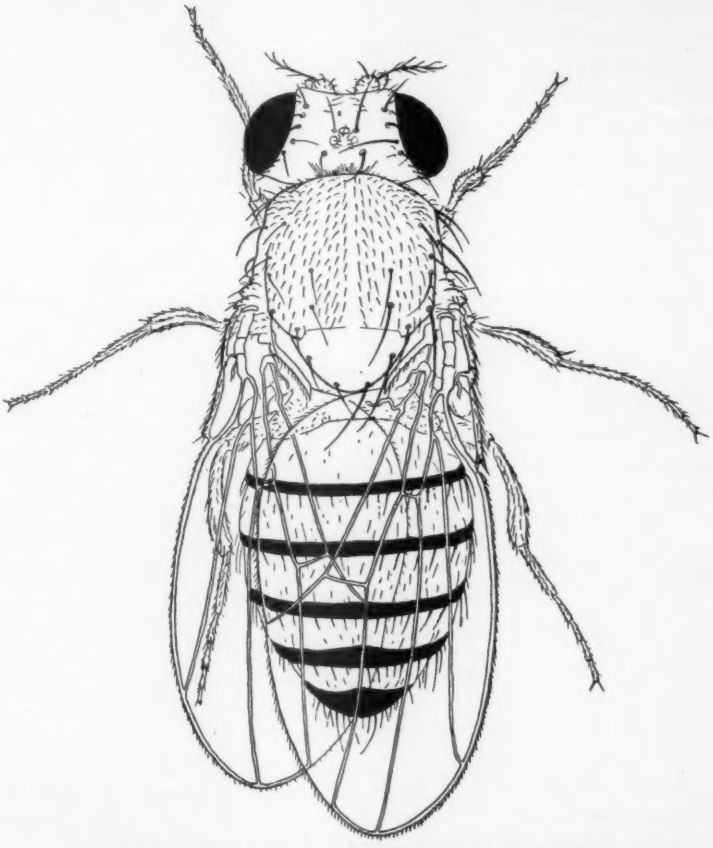

FIGURE 1.—Mosaic female. Left half "Minute," right half wild-type.

the right side. Of the two somewhat larger hairs situated just anterior to the second pair of legs on each side of the median ventral furrow, the left was Minute and the right wild-type. The left wing was markedly shorter than the right, bluntly rounded and with a shortened inter-crossvein dis-

tance. The pair of bristle-like hairs just anterior to the distal costal break were Minute on the left and normal-sized on the right wing.

In the legs there was a slight deviation from the strictly bilateral distribution of the two regions in so far as the larger hairs on the ventral surface of the coxa seemed to be of equal size on both forelegs. From comparison of these hairs in the fixed specimen with the corresponding hairs in wild-type and in Diminished individuals (see p. 64) it was concluded that these hairs were in the mosaic wild-type on both sides. That the rest of the left foreleg was Minute, in contrast to the right foreleg, could be ascertained by comparison of the size of the larger hairs on the femur and the apical and preapical bristles of the tibia. A corresponding difference in size was evident also in the second and third pair of legs and especially striking in the apical and preapical bristles on the tibia of the second pair of legs where they are largest.

In the abdomen the border line separating the Minute and the wild-type region followed strictly the median line on the dorsal surface of the anterior four segments (tergites). This could be ascertained by the difference in size of the larger hairs along the posterior margin of each tergite. The fifth tergite had, in contrast to the others, wild-type hairs both on the left and on the right side. In the remaining part of the abdomen, including the surroundings of the genital and anal openings, the hairs were Minute on the left and wild-type on the right side of the dorsal surface.

On the ventral surface of the abdomen it was evident that the border line followed the median plane strictly, the hairs on the ventral plates being Minute on the left and wild-type on the right side. As regards the anal and genital region it was, by the smallness of the hairs, rather difficult to detect a conclusive difference in size on the ventral side. But after scrutinous examination of the fixed specimen and comparison with wild-type and Diminished flies there seemed to be little doubt that the division in a left Minute and a right wild-type half holds true also for this part of the fly.

Summing up, we find accordingly that we are dealing with a bilateral somatic mosaic in which the division in a left Minute and a right wild-type region is remarkably schematical, the only deviations from the strictly bilateral symmetry being found in the ventral surface of the anterior coxae and in the fifth tergite which are wild-type on both sides.

GENETICAL ANALYSIS OF THE MOSAIC FEMALE

Dominance of the mutant change present in the mosaic

The most plausible explanation of the mosaic female described seemed to be that we were dealing with a somatic mutation to a dominant Minute which had occurred in one of the earliest (probably the first) cleavage

cells, since dominant Minute mutations are of very frequent occurrence in *Drosophila melanogaster*. But another possibility, namely, that the Minute region might be haplo-IV due to somatic elimination of one member of the fourth chromosome pair, was also kept in mind.

Since the mosaic was a normal female with normal external genitalia it was hoped that she might prove fertile. And in view of the bilateral distribution of the Minute and wild-type parts it seemed not unlikely that one ovary, or part of one ovary, might be included within the Minute region. In this case the two alternative hypotheses might be put to a test.

The mosaic was accordingly as a first step backcrossed to $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ males (C. 4732). By this mating we would not only certify genetically that the mosaic was derived from C. 4709 and that no contamination had occurred, but we would also obtain information as to the possible linkage relations of the new mutant to genes distributed over the entire third chromosome.

The mosaic proved fertile, but no Minute flies were obtained during the first nine days' counts. Since, however, delayed emergence is a typical feature of the Minute character complex, the counting was continued, and on the tenth day 3 typically Minute individuals were found. The counting was now continued until all the offspring from C.4732 had hatched. In all 262 individuals were obtained, 16 of which were Minute.

Meanwhile the mosaic female had already on the sixth day been transferred into a new culture bottle (C.4732 a) with the same males, and in this culture the offspring were counted for the ordinary ten days only. The mosaic female which was still vigorous was now drawn and afterwards fixed in alcohol for later examination. The total output of C.4732a were 131 individuals, of which 15 were Minute. In both cultures more than two-thirds of the Minute flies were males, thus indicating that the Minute females had a lower viability than the Minute males in these cultures.

In the following are given the total output of the two cultures, the numbers from C.4732 being printed in ordinary letters, those from C. 4732a in italics. The numbers in parenthesis denote the Minute individuals out of the total in the respective class:

$r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ 21, *19 (2)*, wild-type 52 (6), *26 (1)*; r_u 17 (2), *7 (1)*, $h\ t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ 14, *4*; $r_u\ h$ 5 (1), *8 (2)*, $t_h\ s_t\ c_u\ s_r\ e^s\ c_a$ 8, *6*; $r_u\ h\ t_h$ 2, $s_t\ c_u\ s_r\ e^s\ c_a$ 2; $r_u\ h\ t_h\ s_t$ 2, $c_u\ s_r\ e^s\ c_a$ 3; $r_u\ h\ t_h\ s_t\ c_u$ 6, *5*; $s_r\ e^s\ c_a$ 16 (2), *3 (1)*; $r_u\ h\ t_h\ s_t\ c_u\ s_r$ 4, *3*, $e^s\ c_a$ 2, *1*; $r_u\ h\ t_h\ s_t\ c_u\ s_r\ e^s$ 9, *8*, c_a 32 (3), *11 (3)*; $r_u\ s_r\ e^s\ c_a$ 4, $h\ t_h\ s_t\ c_u$ 4; $r_u\ e^s\ c_a$ 3, *1*, $h\ t_h\ s_t\ c_u\ s_r$ *1*; $r_u\ c_a$ 10 (1), *9 (4)*, $h\ t_h\ s_t\ c_u\ s_r\ e^s$ 12, *2*; $t_h\ s_t$ 2; $r_u\ h\ s_r\ e^s\ c_a$ 6, $t_h\ s_t\ c_u$ 2; $r_u\ h\ e^s\ c_a$ *1*, $t_h\ s_t\ c_u\ s_r$ *2*; $r_u\ h\ c_a$ 4, *7*, $t_h\ s_t\ c_u\ s_r\ e^s$ 9, *2*; $r_u\ h\ t_h\ s_r\ e^s\ c_a$ 1, $s_t\ c_u$ 1; $s_t\ c_u\ s_r$ *1 (1)*; $r_u\ h\ t_h\ s_t\ e^s\ c_a$ 1, $c_u\ s_r$ *1*; $r_u\ h$

t_h s_t c_a 1, *c_u s_r e^s* 2; *r_u h s_t c_u c_a 1, s_r e^s* 1, *1; h s_r e^s c_a 1; r_u c_u s_r e^s* 1; *r_u s_r e^s* 1 (1); *r_u e^s* 1; *t_h s_t c_u c_a* 1.

From this result it is apparent that the supposedly non-virgin mosaic female has at any rate not been fertilized by a Vein brother, since none of her sons and daughters were Vein. Whether she has been fertilized by a wild-type brother is difficult to tell with certainty. The marked numerical preponderance of wild-type non-crossovers in C. 4732 as compared with the corresponding non-crossover class might seem to point in this direction. But an analogous discrepancy is also seen in the two single crossover classes resulting from crossing over within the e^s-c_a region, so it is quite possible that the numerical difference is simply due to the markedly lowered viability in this culture of those flies which are homozygous for roughoid and in addition for the largest number of the other third chromosome recessives present in the test.

Another source of error should also be kept in mind, namely, the possibility of overlapping of generations in C. 4732 where the offspring were counted for 19 days. Thus some of the apparent triple crossovers in this culture may be due to overlapping. A calculation of the linkage values for the different III-chromsome mutant genes involved in the backcross is of no particular interest for our present purpose. In spite of the above-mentioned sources of error such a calculation based on the total data gives values which are in sufficient accordance with the known map distances to show that crossing over has been of normal frequency throughout the third chromosome in this test.

The reappearance of Minute flies in F_1, flies which in every respect resembled the Minute half of the mosaic mother, proved conclusively that at any rate part of the gonads have been included within the mutated part of the mosaic, and, furthermore, that the genotypical change responsible for the Minute character complex here dealt with is inherited as a typical Mendelian dominant.

If, which seems likely, one ovary is included in the mutated region, then we would expect one quarter among the offspring of the mosaic to be Minute (see p. 67), while here only 31 Minutes were obtained as against 362 not-Minute individuals. That this deviation from the expectation might simply be due to the lowered viability of the Minute phenotype was not unlikely since the Minute individuals were examined and found to be small and weak looking in comparison with their non-Minute sibs.

Inheritance as a member of the fourth chromosome linkage group

From the distribution of the Minute individuals in the different crossover classes in the above test, with preferential occurrence in the most

viable classes, it was clear that the new mutant did not belong to the III-chromosome linkage group. And by examination of the Minute individuals it was found that they resembled closely the Diminished, haplo-IV flies described by BRIDGES (1921): They were in comparison with the wild-type of a smaller size throughout, had shorter and more slender bristles, a paler body color, a darker trident pattern on the thorax, larger eyes, and wings which were frequently slightly divergent, blunter and less clear in texture. This in combination with the delayed emergence and the lowered viability made it at once probable that we were dealing not with an ordinary dominant Minute mutation but with individuals which had the fourth chromosome in haploid condition.

Minute males from C. 4732 which were heterozygous for the above-mentioned III-chromosome recessives had in the meantime been mated to a homozygous roughoid female (C. 4772). The result of this mating was roughoid 26, wild-type 29, roughoid Minute 9, Minute 13. Since there is no crossing over in the males, this result confirmed the above conclusion that the Minute mutant did not belong to the third chromosome linkage group. And since there were both females and males in the Minute classes this test proved simultaneously that the Minute mutant did not belong to the first (the sex) chromosome.

In order to test whether the mutant belonged to the II-chromosome group Minute males were also mated to females homozygous for the II-chromosome recessives black (*b*) purple (p_r) and curved (*c*), and F_1 Minute males backcrossed to black purple curved females (C.4798–99). The result of this male backcross was: *b* p_r *c* 192, wild-type 232, *b* p_r *c* Minute 5, Minute 69. This test proves that Minute does not belong to the second chromosome group of linked characters and gives further evidence of the heavy mortality of the Minute flies. This lowering of the viability was particularly extreme in Minute flies which were at the same time homozygous for black purple and curved, a combination which in itself reduces the viability of the fly somewhat (see on this point p. 68).

When it had been proved that Minute showed free segregation with representatives of the first, second and third chromosome linkage groups, it was clear that the dominant character was due to a change in the fourth chromosome since there are in *Drosophila melanogaster* four chromosome pairs only.

This change might either be a dominant mutant gene or lack of one member of the fourth chromosome pair. The latter possibility seemed the more probable in view of the phenotypical similarity of the Minute here studied with Diminished. Additional evidence in the same direction was

also derived from a study of the fertility of the Minute flies. While the Minute males as a rule proved to be of fairly good fertility, sterility or lowered productivity was a typical feature of the Minute females. Among 12 preliminary test cultures where one or (in some cases) more Minute females were mated to unrelated males only six delivered a limited amount of offspring. In larger mass cultures, however, a fair amount of offspring from Minute females could generally be obtained. A Minute stock was therefore maintained by mating Minute flies in mass cultures. It was, however, found advisable to add one or two wild-type sisters in order to get the stock started. An analogous lowering of the fertility is typical also of the Diminished, haplo-IV females.

Deficiency for fourth chromosome recessives

Whether the Minute character change studied was due to a dominant fourth chromosome gene or to haploidy for the fourth chromosome could easily be tested by mating the Minute flies to flies homozygous for the fourth chromosome recessives bent and eyeless, the only representatives of the fourth chromosome group of which stocks were at disposal in the laboratory. As shown by BRIDGES (1917) recessive genes located in the region opposite to a deficient chromosome section manifest themselves in heterozygous condition in flies carrying the deficiency, since their normal allelomorphs are lost or inactivated. If the Minute individuals are deficient for one member of the fourth chromosome pair, we expect accordingly that bent and eyeless will manifest themselves in F_1 of Minute $\times$ bent and Minute $\times$ eyeless respectively.

Females homozygous for black (b, II chromosome) pink (p, III chromosome) and bent (b_t, IV chromosome) were in 10 cultures mated to Minute males [C. 4808 (2 ♀ ♀), 4831–32, 4843, 4860–66].[1] The total output of these cultures were 901 wild-type individuals while only a single Minute male hatched. The latter fly showed the bent character in very extreme form; the wings were folded and the tarsi very much shortened and fused together so that their hairs formed a thick, dark brush distal to the shortened tibia.

[1] One such mating (C. 4809) gave an entirely unexpected result, namely, wild-type 37 ♀ ♀, 8 ♂ ♂; bent 14 ♀ ♀, 4 ♂ ♂. The bent character of these F_1 non-Minute bent flies was very well pronounced, much more so than is the case in the $b\ p\ b_t$ stock flies, where the bent character is poorly developed. When inbred, these F_1 b_t flies behaved as ordinary homozygous b_t. If the Minute type is haplo-IV, as later proved to be the case, the simplest explanation of the above result is the assumption that the P_1 $b\ p\ b_t$ female has been triplo-IV ($b_t\ b_t\ b_t$). When mated to haplo-IV males the expectation is then: wild-type triplo-IV ($b_t\ b_t$ +), wild-type heterozygous for b_t (b_t +), homozygous b_t ($b_t\ b_t$) and Minute-bent haplo-IV (b_t) individuals in equal numbers. The latter type is inviable (see p. 71). Due to extraneous causes the appropriate tests had to be postponed, and in

From this result it was clear that the viability of the F_1 Minute-bent compound was lowered almost to complete lethality, a fact which was also apparent from the dead pupae present in these cultures. When dissected they were found to contain crippled Minute-bent individuals. By aid of the shortened legs with their dark, hairy brush it was also possible to recognize the Minute-bent compound flies through the wall of the puparium. And from all but two of the above cultures Minute-bent flies that were still living, 21 in all, were as a control secured by dissection.

The analogous matings of eyeless female × Minute males (C. 4810–12) had a similar result. In all 482 wild-type flies were obtained as against 13 Minute-eyeless compound individuals (3 ♀ ♀ , 10 ♂ ♂). Thus, also, here the viability of the compound is very markedly lowered. The eyeless character in the Minute-eyeless flies that hatched was not conspicuously more extreme than in ordinary eyeless stock flies. But in individuals that were secured by dissection of Minute-eyeless pupae the eyes seemed on the whole to be slightly smaller than in ordinary eyeless, although the difference was rather inconspicuous here also.

In order to compare the character changes in the Minute-eyeless compound with ordinary eyeless under optimal conditions, Minute-eyeless compound males from C.4811 were mated to eyeless females from stock (C. 4830, 4870). In one of these cultures where a single eyeless female was used 123 eyeless and 6 Minute-eyeless (♂ ♂) were obtained; in the other with three P_1 eyeless females, the output was 251 eyeless and 27 Minute-eyeless (6 ♀ ♀ , 21 ♂ ♂). The eyes were in the Minute-eyeless compound, on the whole not conspicuously smaller than in their ordinary eyeless sibs. In C.4830, however, some eyeless individuals occurred that had such large eyes that they overlapped wild-type so that their homozygous eyeless genotype had to be controlled by testing; no such overlapping individuals were encountered among the Minute-eyeless flies, a fact which involves indirect evidence to the effect that the eyeless character is more pro-

order to secure a stock for later examination the non-b_t flies were searched for individuals which showed the (slight) dominant characteristics described by BRIDGES as typical of the triplo-IV type. It was believed that such were found, and by selection a stock was maintained which continued to throw a percentage of flies with slightly shortened and coarser bristles. When one such fly (third generation) in a mating to Minute (haplo-IV) gave non-Minute bent in F_1, it was believed that the bristle alteration was a reliable somatic indication of the triplo-IV condition. However, when the case was taken up later, it turned out that the bristle alteration mentioned was due to a second chromosome recessive which overlapped the wild-type, and the opportunity to prove the case had accordingly been lost.

The atypical sex ratio in the above culture was found to be due to two sex-linked lethals for which the P_1 female had been heterozygous, one located in the close neighborhood of vermilion and the other about 10 units to the right of forked.

nounced in the compound. That the exaggeration of eyeless is not more distinct may seem natural when it is remembered that the Minute flies have larger eyes than the wild type. The Minute character in other words counteracts the tendency to reduction of the size of the eyes. Still, the very slight exaggeration of the eyeless character contrasts somewhat with other typical cases of exaggeration and may need further investigation (see also p. 80).

The tests presented above proved conclusively that the dominant Minute here dealt with was not due to a point mutation, but to a deficiency which includes the fourth chromosome loci bent and eyeless. The dominance (of Minute) and the exaggeration of the included characters (bent and eyeless) are general deficiency phenomena typical of section deficiency (MOHR 1919) and quite similarly of chromosome deficiency (BRIDGES 1921).

The above results were in all essential respects parallel to those obtained by BRIDGES in matings of Diminished (haplo-IV) to bent and eyeless. As regards the very marked lowering of the viability of the compounds, by BRIDGES considered as another expression of the exaggeration, the following should be added:

Based on the tests presented above (C.4772, 4798–99, p. 69) the viability of Minute as compared with the wild type is 31.4 percent (261 wild-type and 82 Minute, where equality is expected). In some later special matings of wild-type female×Minute males (C. 4953–56) the result was wild-type 421, Minute 160 (67 ♀ ♀, 93 ♂ ♂), that is, the viability of Minute is here 38 percent. To these data may be added those derived from the experiments p. 78 and p. 79, in the majority of which the viability of Minute was far better (C. 4959, 4975, 4977, 5038, 5051–52). These tests comprise 489 wild-type and 353 Minute individuals, where equality is expected, corresponding to a viability of 72.2 percent.

The total data comprise 1171 wild-type as against 595 Minute individuals, that is, the average viability of Minute is 50.8 percent, a value which is strikingly in accordance with that found by BRIDGES for Diminished, namely, 53 percent. But it is evident that the viability of Minute is exceedingly variable, probably as a consequence of slight differences in the culture conditions. In the tests here presented the viability ranges from 22–24 percent (C. 4959, 4798, p. 78) to cases in which the Minute class even outweighs the corresponding wild-type class (C. 5038, 5052, p. 79).

Based on the data presented above (p. 70) the viability of the Minute-bent compound is practically zero as compared with the wild type, while that of the Minute-eyeless compound is 2.7 percent. In analogous tests

with Diminished (haplo-IV) BRIDGES found the corresponding values to be 0.5 and 1.3 percent respectively. As compared with homozygous eyeless, which by itself has a slightly lowered viability, the viability of the Minute-eyeless compound was 8.8 percent (C. 4830, 4870, p. 71). This disproportionate lowering of the viability of the compounds is evidence of exaggeration of the included characters.

Finally, the homozygous lethal action of the Minute dealt with was further evidence of deficiency. Not only was it impossible to reduce the number of wild-type flies by continuous inbreeding, but all the Minute individuals used in the numerous crosses proved to be heterozygous only. It was accordingly regarded unneccessary to control this point further by special tests.

Strictly speaking the above tests demonstrate that the Minute flies are deficient for a section of the fourth chromosome including the loci for bent and eyeless. But in view of the striking similarity with Diminished, not only in somatic, but also in all genetical and in physiological respects (lowered viability, lowered fertility, late emergence, etc.), it was now practically certain that we were not dealing with a section deficiency but with a chromosome deficiency involving one member of the fourth chromosome pair. This could easily be controlled by cytological examination of cells from the Minute individuals.

CYTOLOGICAL DEMONSTRATION OF HAPLOIDY FOR THE FOURTH CHROMOSOME IN THE "MINUTE" FLIES DERIVED FROM THE MOSAIC

Ovaries of Minute females were fixed in strong Flemming's solution and colored with iron-haematoxylin in the ordinary way. A number of satisfactory oogonial plates were found in eight different Minute females, and all had only one small spheric "fourth" chromosome, as had been predicted. Representative photographs and drawings of oogonial plates from four different females are presented in figure 2 and need no further comment. As will be seen, all the other chromosomes are present in duplicate as usual. The result of the cytological examination proves that our Minute mutation is identical with the Diminished, haplo-IV mutant type.

GENERAL CONSIDERATIONS AND CONCLUSIONS

The evidence presented above proves conclusively that the left half of the original mosaic was haplo-IV. Hence, it has in this case been possible to deliver the complete proof that the haplo-IV condition has arisen through somatic elimination resulting in mosaicism. Though the earlier special genetic evidence of somatic elimination of one member of a chro-

mosome pair is in Drosophila fully convincing (see Introduction) the present case fills a gap in so far as it represents the first in which the elimination of a chromosome in somatic cells has been proved by complete breeding tests and cytological demonstration. Thus, it puts the proof of somatic elimination and mosaicism on the same level as those previously delivered for chromosome aberrations due to non-disjunction during the maturation of the germ cells. As regards some of the earlier mosaics which were interpreted as Diminished (haplo-IV) the possibility could not be excluded

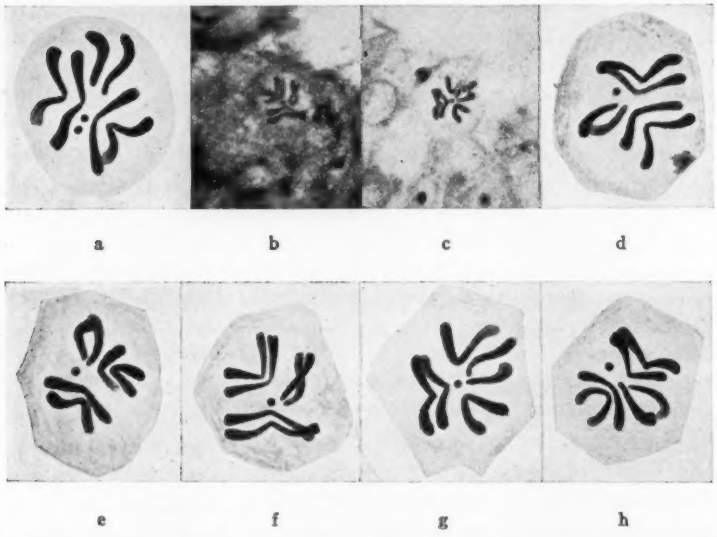

FIGURE 2.—Oogonial plate from 2n *Drosophila melanogaster* female, for comparison. b–h oogonial plates from five different Diminished (haplo-IV) females derived from the mosaic. Note: One small round fourth chromosome only; b and c photographs, about 2000×;[2] d–h drawings, Zeiss Comp. Oc. 12, Obj. 1.5 mm Apochromat N.A. 1.30, drawn at table level by aid of Abbés apparatus, Tube length 160 mm; f same as b; g same as c.

Strong Flemming's solution; iron-haematoxylin.

that they were due to somatic mutation to a dominant Minute in one of the early cleavage cells. This source of error is here eliminated.

The bilateral symmetry of the mosaic studied makes it practically certain that the elimination of one member of the fourth chromosome pair has occurred during the first cleavage division. The slight deviations from the

[2] For the preparation represented in figures b and f as well as for the photographs the author is indebted to kand. real. T. QUELPRUD.

bilateral symmetry (coxa of first leg, fifth tergite) are due to wandering of the cleavage cells during the developmental stages prior to the formation of the imaginal discs (compare the detailed analysis of the distribution of parts in sex mosaics by STURTEVANT 1929).

The genetical test of the mosaic proves that one ovary (or part of one ovary) is derived from one cleavage cell while the rest of the germinal tissue is derived from another. Hence the germinal tract in Drosophila cannot be derived from one and the same cleavage nucleus. In this respect the

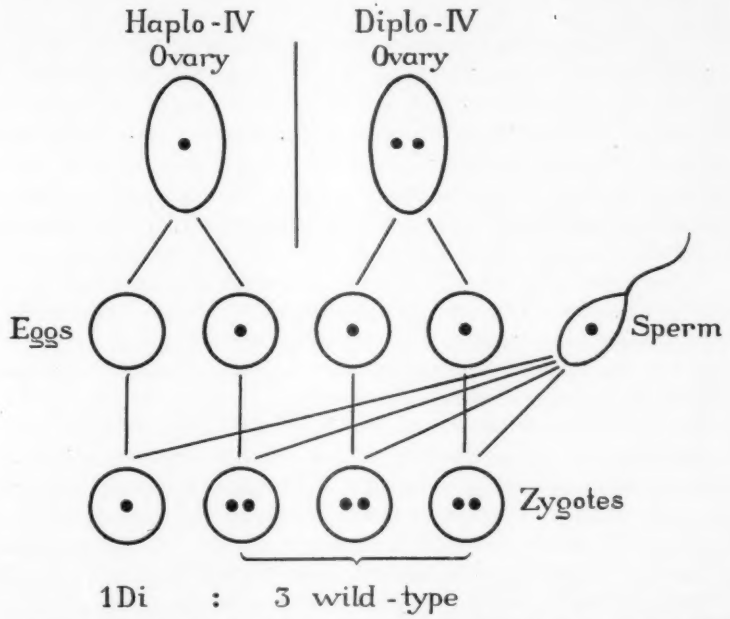

FIGURE 3.—The result of mating the Diminished mosaic to normal males, if one entire ovary is haplo-IV and the other diplo-IV.

genetical evidence is parallel to that obtained in the analysis of a somatic mosaic (MOHR 1923) and in accordance with HUETTNERS (1923) cytological findings that more than one cleavage nucleus gives rise to the primordial germ cells.

In this connection it is of interest to decide whether one entire ovary or only part of an ovary has been included within the haplo-IV part of the mosaic. If one entire ovary is included, we expect half of the eggs from this

ovary to be nullo-IV and the other half haplo-IV. The other ovary will deliver haplo-IV eggs only.

Hence if both ovaries deliver an equal amount of eggs, we expect, when these eggs are fertilized by normal, haploid sperms, to obtain non-Diminished and Diminished offspring in a 3:1 ratio (see figure 3). But this expectation will only be fulfilled if the viability of Diminished is on a par with that of the non-Diminished type.

In the test of the mosaic we obtained 362 non-Diminished and 31 Diminished flies. One third of the non-Diminished individuals are on the above assumption derived from the haplo-IV ovary. This ovary has accordingly delivered 121 non-Diminished and 31 Diminished flies. This deviation from the expected 1:1 ratio is readily explained by the markedly lowered viability of the Diminished phenotype. Based on the above data the viability of Diminished is in this test about 26 percent, a value which lies within the limits actually obtained in ordinary out-crosses of Diminished (see p. 64). And in the test of the Diminished mosaic the viability of the Diminished offspring was still more reduced by the simultaneous presence of numerous third-chromosome recessives which are expected to reduce the viability more in the Diminished than in the corresponding non-Diminished classes. From the numerical data of the case we arrive accordingly at the conclusion that one entire ovary in the mosaic was haplo-IV and the other diplo-IV, a conclusion which falls into line with the bilateral distribution of parts in the mosaic female.

In the test of the mosaic it was a striking fact that Diminished offspring did not emerge until the tenth day of counting. This result is exceptional, since in ordinary out-crosses of Diminished the first Diminished offspring generally appear on the third or fourth day and never so late as was here the case. This seems to indicate that the egg development or the egg laying is retarded in the Diminished phenotype so that in the mosaic the eggs from the normal ovary have been deposited earlier than those from the haplo-IV ovary. This in combination with the slow development typical of the Diminished zygote would account for the very late emergence of the Diminished offspring of the mosaic in the first test culture (C.4732 p. 67). Special tests in order to compare the time of egg laying in Diminished and in non-Diminished flies have not been carried out.

The fact that the mosaic female in the first culture bottle (C. 4732) gave 262 non-Diminished and only 16 Diminished flies, the first of these appearing on the tenth day of counting, while in the other culture (C. 4732a, p. 67) 131 non-Diminished and 15 Diminished flies were obtained, the first Diminished individuals here appearing on the fourth day, lends

support to the above assumption. In this case it would be more correct to use the data from the ten days' counts of C. 4732a only for deductions concerning the constitution of the ovaries of the mosaic female.

Of the 131 non-Diminished offspring in C. 4732a one third of the 131 non-Diminished flies plus 15 Diminished individuals are expected to be derived from the haplo-IV ovary. This ratio, 44:15, corresponds to a viability of Diminished of 34 percent, a value that is in still better accordance with the known viability of Diminished in ordinary out-cross tests.

SUMMARY

The possibility of delivering the combined genetical and cytological proof of somatic elimination of a chromosome as a cause of mosaicism is discussed.

A female mosaic is described. The entire right half plus the ventral surface of the left first coxa and the left half of the fifth tergite were wild-type; the rest of the individual exhibited a typical "Minute" character complex. Except for the slight deviations mentioned, the distribution of Minute and of wild-type parts was strictly bilateral both on the dorsal and on the ventral surface.

The mosaic was fertile, and the Minute character complex reappeared unchanged in a proportion of the F_1 flies. By appropriate genetical tests it was demonstrated that this dominant Minute was not due to an ordinary gene but to deficiency for one member of the fourth chromosome pair.

Cytological examination of ovaries from the Minute females proved that their cells contained only one member of the fourth chromosome pair as had been predicted. Hence the Minute mutant here dealt with is identical with the Diminished (haplo-IV) type.

The evidence presented proves conclusively that the elimination of the fourth chromosome must have occurred in one of the earliest, in all probability the first, cleavage divisions of the egg from which the mosaic developed. And the case represents the first in which somatic elimination of a chromosome (resulting in mosaicism) has been proved by combined genetical analysis and cytological demonstration.

Based on the numerical data of the case it is concluded that in the mosaic one entire ovary was haplo-IV and the other diplo-IV. Hence the case involves genetical evidence to the effect that in Drosophila more than one cleavage cell goes into the formation of the germ tract.

Some data on special peculiarities of the black purple curved Diminished and the Diminished-eyeless phenotype are presented and discussed in the appendix.

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APPENDIX

Notes on the black purple curved Diminished and the Diminished-eyeless phenotype

In the male backcross of Diminished flies heterozygous for the II-chromosome recessives black purple and curved, there was a very extreme lowering of the viability of the $b\ p_r\ c\ D_m$ class (C. 4798–99, p. 69), and the very few $b\ p_r\ c\ D_m$ flies obtained were all males. A certain lowering of the viability of the $b\ p_r\ c$ phenotype in comparison with the wild-type was also apparent in these cultures. But this fact seemed not sufficient to account for the very disproportionate lowering of the viability when $b\ p_r\ c$ flies were at the same time Diminished.

The male backcross was accordingly repeated (C. 4959). Though not quite as marked, the result was similar, the following offspring being obtained: $+90, b\ p_r\ c\ 77, D_m\ 20, b\ p_r\ c\ D_m\ 4$ (all males). The latter individuals were small, weak and had a humid body surface so that they got stuck

while emerging from the puparium. It was a general feature that the proximal tarsi were in these individuals very short and clumsy, while the distal ones were fused and reduced into a very thin thread without claws. In the forelegs these malformations caused irregularities in the sex combs which were also reduced in size. Though the tarsi are also in ordinary Diminished flies thinner than in the wild-type, these alterations in combination with the extreme lowering of the viability were so striking that the question arose whether we were here possibly dealing with exaggeration of non-included characters. In the few cases in which the wings of these *b* p_r *c* D_m flies were unfolded, they were small, but otherwise like those of ordinary curved individuals.

A few tests were arranged in order to look into the matter. Diminished males heterozygous for purple were in two cultures backcrossed to purple females (C. 4975, 5038). The output were in the two cultures: wild-type 66+59, p_r 64+49, D_m 32+ 71, p_r D_m 14+58, respectively. In some young p_r D_m individuals the p_r character seemed perhaps to be slightly more transparent than in ordinary purple flies of the same age. But this seemed simply to be connected with the larger and more roundish eye form typical of the Diminished individuals. As seen from the totals, there was in one of the cultures a certain lowering of the viability of the p_r D_m flies, but in the other, where the viability of Diminished was extraordinarily good, the p_r D_m class even outweighed the corresponding p_r class.

Analogous tests were also carried out with curved (C. 5051–52) and gave: wild-type 96+62, *c* 92+80, D_m 80+79, *c* D_m 49+31. The curved character failed to show any modification in combination with Diminished. The tarsi were normal. But it will be noticed that there is a distinct lowering of the viability of the *c* D_m phenotype, although this lowering is of an entirely different order from that typical of the compounds of Diminished with the included IV-chromosome recessives.

An F_2 was also raised from a female heterozygous for *b* p_r *c* by males which in addition were Diminished with the following result (C. 4977): +116, *b* p_r *c* 47; *b* 6, p_r *c* 3; *b* p_r 5, *c* 7; *b c* 2, p_r 1; D_m 71, *b* p_r *c* D_m 3; *b* D_m 4; *b* p_r D_m 1; *c* D_m 5. The *b* p_r *c* D_m flies, all males, had thread-like fused tarsi and got stuck while emerging. The other D_m classes showed no disproportionate modifications. Finally, two black Diminished males from C. 4977 were mated to *b* p_r *c* females and gave (C. 5008): *b* 106, *b* p_r *c* 121, *b* D_m 41, *b* p_r *c* D_m 3 (all males of the type described). The *b* D_m flies were slightly lighter and more brownish than ordinary black individuals, but not more so than would be expected in view of the light body color typical of Diminished.

Judging from the above tests the very low viability of the $b\ p_r\ c\ D_m$ phenotype must be due to an additive effect of the lowering of the viability caused by these II-chromosome recessives separately in combination with Diminished. And the disproportionate modification of the $b\ p_r\ c\ D_m$ phenotype must correspondingly be due to a summation of latent tendencies which are not by themselves strong enough to produce manifest character changes. In view of such evidence a certain caution seems advisable when it is a question of considering lowering of the viability as evidence of exaggeration.

The Diminished-eyeless compound described above (p. 71) contrasts with other typical cases of exaggeration in so far as the exaggeration of the eyeless character is almost negligible in spite of the very extreme lowering of the viability of the compound. This situation seems still more surprising if PATTERSON and MULLER (1930) are right in considering a new IV-chromosome mutation which reduces the size of the eyes as a dominant allelomorph of eyeless. In this case "dominant eyeless" would, according to the statements given, have to be considered as a more extreme allelomorph than ordinary eyeless. In view of the best analyzed cases of exaggeration we would then have expected more extreme character changes, analogous to those present in "dominant eyeless," also in the Diminished-eyeless compound, since the analysis of the other cases of deficiency has led to the conclusion that deficiency for a given locus represents the final step, the bottom, so to speak, of the series of mutative changes that are possible in this particular locus (see on this point MOHR 1929).

However, the evidence for "dominant eyeless" being an ordinary point mutation allelomorphic to eyeless seems not fully convincing, and PATTERSON and MULLER also express themselves with a certain reservation on this point. It is also possible that the apparent inconsistency mentioned above may bear relation to the fact that we are here dealing with a chromosome deficiency. It may well be that a more thorough comparison may show that the exaggeration phenomena in section deficiency and in chromosome deficiency though analogous in main features, may nevertheless show typical differences that are not yet recognized.

INVERSION IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

Many genetic factors suppressing crossing over in *Drosophila melanogaster* have been described and their locations in the three large chromosomes determined (MORGAN, BRIDGES and STURTEVANT 1925). The first case of this kind was encountered by A. H. STURTEVANT (1913). A genetic factor located near sooty in the third chromosome inhibited crossing over in that region when present in heterozygous condition. The factor was therefore considered dominant and was represented by the symbol *C* (crossing over suppressor). However, a fly homozygous for *C* showed normal crossing over values. MULLER found the same crossing over suppressor and analyzed its effects (MULLER 1916). STURTEVANT later reported a case (1919), found in a stock of wild flies collected in Nova Scotia, in which two separate crossover suppressors were present simultaneously, one in each limb of the second chromosome. The one in the left limb was found to suppress practically all crossing over in the half of the chromosome to the left of purple eye color and the one in the right limb had the same effect on the region to the right of purple. For this reason they were named C_{IIL} and C_{IIR} respectively. The number II designates the linkage group, L the left and R the right limb of the chromosome. As in the first case found, no suppression of crossing over occurred when these factors

were present in homozygous condition. A similar case of double crossing-over suppressors was reported in the third chromosome by PAYNE (1924). During the course of the breeding experiments with PAYNE'S suppressors (C_{IIILP} and C_{IIIRP}) a lethal arose by mutation in the left limb and another in the right limb. Several other cases have been reported where suppression of crossing over occurred in definite regions or along the entire length of the first, of the second or of the third chromosome. The crossover suppressors reported by GOWEN and GOWEN (1922) for Drosophila, and by BEADLE (1930) for maize, which inhibit crossing over in all chromosomes, are probably of a different character.

The experiments reported in this paper were conducted on suppressors found by L. WARD (1923). She found flies which showed a new character, Curly wings. They were isolated and found to breed true for the character, which proved to be a dominant, located in the second chromosome. The breeding true, however, was due to "balanced lethals," such as those found by MULLER (1918). As a rule, neither homozygous Curly nor the homozygote of the homologous chromosome survived. Curly (symbol $= C_y$) was found to be linked with cinnabar-2 (c_n^2) eye-color, an allelomorph of cinnabar, slightly duller in appearance. It soon became apparent that the presence of Curly cinnabar-2 suppressed crossing over along the entire second chromosome. High temperatures (30° C and 14° C) were found to increase markedly the crossing over above the small amount occurring at the standard temperature of 25° C. By such crossing over the Curly and the cinnabar-2 were separated. Heterozygous Curly without cinnabar-2 gave crossing over in the right half but not in the left in which it is located. Heterozygous cinnabar-2 permitted normal crossing over in the left limb but inhibited it in the right limb in which its locus is. It was assumed that the suppression of crossing over was not due directly to the C_y and c_n^2 genes but to associated crossing over factors, namely, $C_{IIL}\, C_y$ with C_y and $C_{IIR}\, C_y$ with c_n^2.

A consistent explanation of the phenomenon of crossover suppressors was advanced by STURTEVANT (1926). From work with C_{IIIB}, a factor which he showed to be allelomorphic to his C_{III} he reached the conclusion that the inhibition of crossing over was due to an inversion of a section of the chromosome. In the case of C_{III} a section at the end of the right limb of the third chromosome, beginning a few units to the right of stripe, showed an inverted order for the genes within it. STURTEVANT also suggested that the explanation of certain rare crossovers in a fly heterozygous for a crossover suppressor was that the chromosomes had synapsed with one chromosome inverted in relation to the other. In this case the genes of

the inverted section would be opposed to the corresponding genes in the normal chromosome in correct order. But only double crossovers would then survive, since singles would produce either too large or too small a chromosome with consequent derangement of development.

The experiments described below show that the same explanation of inversion holds for the C_{IIR} with cinnabar-2. The chromosome containing the different order of genic material is then utilized for obtaining information upon certain aspects of crossing over.

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ANALYSIS OF $C_{IIR}\ C_v$

Experimental

Flies of the constitution $C_{IIL}\ C_v\ C_v\ C_{IIR}\ C_v\ c_n^2\ s_p/a_l\ b\ p_r\ c_n\ v_g\ s_p$ (a_l equals aristaless; b equals black body color; p_r equals purple eye color; c_n equals cinnabar eye color; v_g equals vestigial wings; s_p equals speck, pigment spot at base of wings; for the remainder of this paper the symbols C_L and C_R will be used instead of the longer forms $C_{IIL}\ C_v$ and $C_{IIR}\ C_v$.) were obtained from C. B. BRIDGES and mated to black-purple-Lobe-c and black-purple-vestigial females. F_1 females $C_L\ C_v\ C_R\ c_n^2\ s_p/b\ p_r\ L^c$ (L^c equals lobe eye, piece of eye missing) and $C_L\ C_v\ C_R\ c_n^2\ s_p/b\ p_r\ v_g$ respectively were backcrossed to the second-chromosome multiple recessive "albasp" (an abbreviation for the homozygous II-chromosome multiple recessive stock: aristaless-black-purple-cinnabar-vestigial-arc-speck) males, for the purpose of studying the extent of the suppression of crossing over throughout the length of chromosome II. The results are given in tables 1 and 2. The recombination percentages obtained in experiment 1 were: $C_v - b(1)$, 0.0; $b - p_r$ (2), 0.4; $p_r - C_R(3)$, 0.04; $C_R - s_p$ (6), 0.04. Within the C_R-inversion there were also six double crossovers (4, 5) which will be discussed below. In experiment 2 the recombination percentages were: $C_v - b(1)$, 0.0; $b - p_r$ (2), 0.5; $p_r - C_R(3)$, 0.08; $C_R - s_p$ (6), 0.01; included doubles (4, 5), 5.0. The total crossing over was thus less than one percent of the normal amount.

Some of the crossovers obtained could not be mated successfully. Of those that produced offspring, the following flies were shown to carry C_R:

vestigial-cinnabar-2-speck, black-purple-cinnabar-2-speck, Curly-Lobe-c-cinnabar-2-speck, and Curly-vestigial-cinnabar-2-speck. In $C_y\ v_g\ c_n{}^2\ s_p$ the Curly did not show on account of the short wings of vestigial, but was revealed by testing. These four types of flies arose through rare double crossing over within the inverted section (4, 5). Curly-purple-Lobe-c, Curly-purple-vestigial and black-purple did not have C_R.

TABLE 1*

$\lceil C_y \rceil$ $\lceil c_n{}^2 \rceil$ s_p ♀ × "albasp" ♂

1 *b* 2 p_r 3 4 L^c 5 6

0	2	3	6	4,5	T
3926+3911	10+20	1+2	2+1	3+3	7879

* Explanation of tables: In the diagrams heading each table the chromosome bearing the leftmost mutant gene is placed above its homologue, with a horizontal line between them. The horizontal bracket for the upper or lower chromosome signifies presence of inverted section throughout the region covered by the bracket. Two such bars opposite each other, one for the upper, one for the lower chromosome, mean that the fly is homozygous for the inversion. Small Arabic numerals, placed below the dividing line and between the symbols for the genes, designate the section in which crossing over is followed. In the table the symbol 0 stands for the non-crossover classes. Similarly the numerals in the table headings designate the type of crossing over of the flies entered below. Thus, "4, 5" indicates double crossovers—one crossover in region 4, the other in region 5. Of the two complementary classes for each crossing-over section, the class which carries the leftmost gene is given first or above. T at end of table stands for total number of flies counted in the experiment. In those tables in which no temperature is stated the flies were in an incubator at 25°C ± 0.5.

TABLE 2

$\lceil C_y \rceil$ $\lceil c_n{}^2 \rceil$ s_p ♀ × "albasp" ♂

1 *b* 2 p_r 3 4 V_g 5 6

0	2	3	3,6	4,5	T
3843+3830	19+20	0+5	0+1	3+2	7723

Experiments were then undertaken to find which genes lay within and which outside of the inverted section, if such was the nature of the suppressor, as assumed. By crossing over in the $\overline{|C_y|}\ \overline{|L^c\ c_n{}^2|}^1\ s_p/+$ stock a male was obtained which was $\overline{|L^c\ c_n{}^2|}\ s_p/+$. This male was mated to a $\overline{|C_y|}\ \overline{|c_n{}^2|}/+$ female (stock) and the F_1 ♀ $\overline{|C_y|}\ \overline{|c_n{}^2|}/\overline{|L^c\ c_n{}^2|}\ s_p$ females were crossed to black-cinnabar-2-speck males. Crossovers appeared in this cross, because the

[1] The square horizontal bracket is used to designate loci within an inversion.

mother was homozygous for the crossover suppressor linked with cinnabar-2. A double-crossover fly of the composition $\overline{|C_y|}\ \overline{|L^e\ c_n{}^2|}/b\ \overline{|c_n{}^2|}\ s_p$ was then mated to black-cinnabar-2-speck. This cross produced $b\ \overline{|L^e\ c_n{}^2|}/b\ \overline{|c_n{}^2|}\ s_p$ males, which were mated to $\overline{|C_y|}\ \overline{|v_g\ c_n{}^2|}\ s_p/b\ \overline{|L^e\ c_n{}^2|}$ females from table 2. Daughters from this cross were backcrossed to "albasp" males and gave the offspring recorded in table 3. In the diagrams in the headings of tables 3, 4, 5, 6 and 8 the mothers were homozygous for $c_n{}^2$ and hence the symbols have been omitted from both homologs.

TABLE 3

C_y v_g s_p ♀ × "albasp" ♂

1 b 2 L^e 3 4

0	2	3	4	2,3	2,4	3,4	T
1726+1836	226+220	121+116	1009+997	1+6	54+59	9+7	6387

When the data of table 3 are calculated by the ordinary methods, the order of genes is as follows: C_y, b L^e, v_g, s_p. The corresponding recombination percentages are: $C_y - b$, 0.0; $b - L^e$, 8.9; $L^e - v_g$, 4.1; $v_g - s_p$, 33.4. This order definitely proves that $C_R(C_{IIR}\ C_y)$ is an inversion, for the normal order is b, v_g, L^e, s_p (BRIDGES 1921). Both L^e and vestigial are included in the inversion, as shown by the fact that vestigial, which is normally to the left of Lobe-c, is now to the right; that is, the region containing both has been reversed.

In table 1 two flies are listed as black-purple-cinnabar-2-speck. When they appeared their genetic constitution could not be ascertained at first, and they were temporarily classified as having a new eye-color mutation because the combination of purple-cinnabar-2 was not then known to have a different eye color from purple-cinnabar, resembling purple more closely than the light orange color of $p_r\ c_n$. Further tests, and backcrosses to cinnabar-2 and purple, showed these two genes to be present. As a result of having obtained the crossover suppressor with purple the above experiments were repeated with this additional locus followed. Flies homozygous for black-purple-$\overline{|}$vestigial-cinnabar-2$\overline{|}$ were obtained and crossed to $\overline{|}$Lobe-c-cinnabar-2$\overline{|}$-speck. F_1 females, homozygous for C_R and for $c_n{}^2$, were backcrossed to "albasp" males. The results are recorded in table 4. The recombination percentages are: $b - p_r$, 2.6; $p_r - L^e$, 7.9; $L^e - v_g$, 4.4; $v_g - s_p$, 30.4. The distance of 2.6 for black-purple may appear to be significantly below its standard value of 6, but later experiments conducted on

a larger scale gave consistently values between 4 and 5. None of the other values deviate beyond the usual amounts.

TABLE 4

					b	*pr*	*vg*							
♀ × "albasp" ♂					1	2	*Le*	3	4	*sp*				
0	1	2	3	4	1,2	1,3	1,4	2,3	2,4	3,4	1,2,4	1,3,4	2,3,4	T
1895	48	226	139	933	6	3	23	5	38	8	3	1	0	
2155	67	230	144	1076	5	3	21	3	44	4	1	0	1	7082

While it is obvious that the C_R inversion included vestigial and Lobe-c, and to some extent genes outside this section, it is not certain that the break occurred far enough to the left to include the cinnabar locus. However, if the locus of cinnabar lies outside the inversion, then some crossing over ought to occur between it and the inversion. None has been detected in the large scale experiments presented in this paper nor in the large volume of miscellaneous work carried out with the Curly stock by BRIDGES and others. It is known, from the work of STURTEVANT on C_{IIIB}, that crossing over can occur, and in considerable amount, between the locus of the spindle fiber and the inversion. In the case of the second chromosome, the locus of the spindle fiber has been found to be very close to purple (BRIDGES 1919, STURTEVANT 1919) and to the right (DOBZHANSKY 1930). Furthermore, in the high-temperature experiment reported later in table 7, the amount of crossing over between purple and the C_R inversion rose to the high value of 2.4 percent. The total absence of crossing over between c_n^2 and C_R under this and all circumstances makes it very probable that the break came to the left of cinnabar. Hence the locus of c_n^2 in the inversion is transposed to a position to the right of Lobe-c and vestigial and near the intact terminal section of normal chromosome.

On the assumption that the break came to the left of the cinnabar locus, we may evaluate the probable length of the inverted section. Assuming that the break did not occur to the left of the middle of the purple-cinnabar interval, we may assign 1.5 as the probable maximum length of the section normally to the left of cinnabar. The cinnabar-vestigial and the vestigial-Lobe-c intervals of 9.5 and 5.0 (MORGAN, BRIDGES and STURTEVANT 1925) are both included. In the homozygous inversion data (tables 3,5) the purple-Lobe-c interval is about 10.5. All of this is within the inversion except the amount by which the break came to the left of cinnabar.

If we assume this to have been 1.5 units, then 9.0 units are included. This gives a total of 1.5+9.5+5.0+9.0=25 units. In view of what was said above, the actual length of the inversion may be smaller than this figure, although the difference cannot be great.

STURTEVANT's hypothesis provides a simple explanation of the way in which the genes for vestigial and Lobe-c crossed into the inverted section. When C_R is present in heterozygous condition the following situation occurs at synapsis:

$$\begin{array}{lc} & b\ p_r\ c_n\ v_g\ L^c \quad s_p \\ \text{Normal} & \text{———————} \\ C_R & \text{———————} \\ & b\ p_r\ L^c\ v_g \quad c_n^2 \end{array}$$

This type of synapsis does not allow crossing over to take place in the inverted section of the chromosome because the genes in that region are not opposite their homologs. But occasionally synapsis occurs in such a way that the genes in the inverted section are opposite their allelomorphs in the other chromosome while the remainder of the chromosome is mated inversely, as represented by the following diagram:

$$\begin{array}{lc} & b\ p_r\ c_n\ v_g\ L^c \quad s_p \\ & \text{———————normal} \\ C_R & \text{———————} \\ & s_p\ c_n^2\ v_g\ L^c\ p_r\ b \end{array}$$

The presence of an inversion in one chromosome at synapsis means that when one section is opposed by its allelomorphic genes the remainder is not. It seems that chromosomes tend to synapse in such a manner as to have the larger part opposed by allelomorphic genes. Occasionally, however, the inverted fraction synapses homologously, causing the remainder to be inverted. The frequency of such synapses would probably be higher the longer the inversion or the larger the ratio of the inverted section to the remainder. With this type of synapsis it is apparent that single crossovers within the inversion would produce two irregular chromosomes, one too short and the other too long in relation to the normal second chromosome. Each of these long chromosomes would be a "deficiency" for the region to the right of the inversion and a "duplication" for the region to the left. The short chromosomes would be deficient for the left region and duplicated for the right region. There is reason to believe that a fly possessing either of these chromosomes would not survive, and none have been found. Every crossover into the inverted section was either Curly-

Lobe-c-cinnabar-2-speck or Curly-purple-vestigial-cinnabar-2, both double crossovers.

The data show that the number of included double crossovers was 11 in a total of 15,607 flies (tables 1 and 2), a frequency of one in 1420. Since these flies are double crossovers, the frequency of the corresponding single crossovers which perish should be much higher. For this section of 25 units the number of single crossovers would be expected to be about 50 times as frequent as the number of double crossovers. This means that the expected frequency of single crossovers is one in 28 eggs, or 3.5 percent. Such a figure would indicate that the number of cases of inverse synapsis is very high, roughly one-seventh of the total cases, since 3.5 is about one-seventh of the normal frequency of singles. An accurate evaluation of the frequency is impossible because inverse synapsis, by causing the original inversion to be opposed by allelomorphic genes, produces on either side of that section non-allelomorphic sections of genes. Such sections would behave like inversions and therefore interfere with crossing over even in the inverted section where synapsis is in correct sequence. Such a condition would necessitate assigning still greater values to the frequency of inverse synapsis.

Conclusions and discussion

The above data show that we are dealing here with a chromosome about a quarter of which is inverted and which therefore presents a different order of genic content. That such inversion does not affect the external appearances of the fly had been demonstrated in many other cases as well as in this instance. The genes are all represented in the normal ratio, or in the normal "genic balance."

The C_R inversion happens to be situated near the center of the chromosome. It differs in that respect from the inversion reported by STURTEVANT which is situated at the end of the third chromosome. A central inversion has been found by SHLAER (unpublished) in the third chromosome, but it contained only one known gene, c_d^2, and was therefore less useful than the present inversion.

Experiments reported by BRIDGES (1915, 1919), PLOUGH (1917, 1921), and by BRIDGES and MORGAN (1919, 1923) show that the centrally located regions behave differently with respect to the influence of age and temperature on crossing over than the regions located at the ends. DOBZHANSKY (1930) presents evidence corroborating the view that the relation between the genetic and cytological maps of these central positions in one of the V-shaped chromosomes is also different. STERN's study of the age

effect upon crossing over in chromosome I confirms the view of BRIDGES that the presence of the spindle-fiber attachment is correlated with this special sensitivity of the central regions of chromosomes II and III. It therefore seemed likely that a study of a chromosome containing an inversion near the spindle fiber in the center would supply some information about crossing over in relation to the above mentioned phenomena.

1. A study was made of the possible modification of crossing over in one arm of the second chromosome, while that in the other arm is inhibited. STURTEVANT (1919) and PAYNE (1924) had shown that in general there was no effect of one arm upon the other. But it seemed desirable to see whether high or low temperatures would have any effect upon this relationship. Some of the data of PAYNE and of DOBZHANSKY (1930) seemed to indicate a rise in crossing over values in one arm when none occurred in the other, or, as in DOBZHANSKY'S case, when some interference occurred in the other.

2. Both high and low temperatures, as mentioned above, greatly increase crossing over in regions that lie in the middle of the second chromosome. The more distant a region is from the center, the less is its crossover value affected by the temperature change. In fact, beyond fifteen units to the right and probably also to the left of purple, only slight influence of temperature on crossing over values has been observed. It is therefore of interest to find how a section, which is normally so far removed from the spindle fiber that its crossover values are practically unaffected by high and low temperatures, would react to the same change of environment when transferred by inversion to a point nearer the spindle fiber. Such a section is the part of the chromosome lying normally to the right of Lobe-*c* but lying between purple and Lobe-*c* in the inverted chromosome. Temperature effects were therefore studied at 25° C, 30° C, 16.5° C and 14° C. Controls were also run simultaneously upon the normal chromosome. These experiments should decide whether the effect of temperature on crossing over is a function only of the genes between the two points involved, or of the proximity of a section to the point of spindle-fiber attachment.

3. Although the published data on coincidence in the second or third chromosome are very scanty they seem to show that the general mechanism shown by WEINSTEIN (1918) to be at work in the first chromosome cannot be applied to either of the V-shaped ones as a whole. In fact, the statement made by BRIDGES and MORGAN (1923) that the symmetrical behavior of crossing over in each arm of the two large autosomes seems to indicate that synapsis and crossing over begin either at both ends of the

arms and proceed from there to the center, or that they start at the center and proceed simultaneously to both ends, necessitates an analogy between the X chromosome and only one arm of the V.

EFFECT OF CROSSING-OVER IN ONE ARM UPON ITS OCCURRENCE IN THE OTHER AT 25°, 30° AND 16° C

Experimental

The gene for Curly is linked with a genetic factor which suppresses practically all crossing over to the left of purple. For this reason it was used in heterozygous condition in flies which were also heterozygous for the genes, black, purple, Lobe-*c*, vestigial and speck. All of these except black are located in the right arm of the second chromosome. The flies were homozygous for the right arm inversion and therefore gave free crossing over.

Females of the constitution black-purple-vestigial-cinnabar-2 were mated to $C_v\ L^c\ c_n^2\ s_p/+$ males. The F_1 Curly-Lobe-*c* females, homozygous for C_R, were backcrossed to "albasp" males. The results obtained at 25° C are given in table 5. A year later this experiment was repeated using

TABLE 5.

$\overline{|\ C_v\ |}$ $\overline{|\ L^c\ |}$ s_p

1 *b* 2 p_r 3 $|\ 4v_g\ |$ 5 ♀ × "albasp" ♂

0	2	3	4	5	2,4	2,5	3,4	3,5	4,5	3,4,5	T
1567	3	290	143	915	0	0	2	76	11	0	
1518	4	243	149	873	1	1	1	67	11	1	5876

different stocks. Females $C_v\ p_r\ v_g\ c_n^2$/"albasp" were mated to $a_l\ b\ L^c\ c_n^2\ s_p$/"albasp" males and F_1 Curly-Lobe-*c* females, homozygous for C_R, backcrossed to "albasp" males. This cross was conducted also at 30° and at 14.5° C. The results are given in table 6. The recombination percents obtained from these two experiments at 25° C show very good agreement, as can be observed from table 10, where the values at both high and low temperatures are also given.

A control experiment with normal left arms and with crossing over prevented in the right arms by heterozygous C_R was also performed. Flies homozygous for black-purple-cinnabar-2-speck were mated to aristaless-dumpy males (dumpy equals dumpy wings, symbol d_p), and the F_1 females backcrossed to males homozygous for aristaless-dumpy-black-

TABLE 6

a_l b L^s s_p ♀ × "albasp" ♂

1 C_y 2 $3p_r$ 4 $5v_g$ 6

T°C	0	3	4	5	6	3,4	3,5	3,6	4,5	4,6	5,6	3,4,6	4,5,6	T
14.5°	1009	9	203	84	464	0	1	1	3	30	3	0	0	
	1033	10	187	80	481	1	1	7	2	42	5	1	0	3657
25°	296	1	47	12	175	0	0	0	1	12	2	0	0	
	305	0	48	34	178	0	0	2	0	14	3	0	1	1131
30°	523	5	94	36	362	0	0	9	3	45	12	0	3	
	516	7	95	48	295	0	0	2	3	56	15	1	0	2130

purple-cinnabar-vestigial-speck ("aldpbasp"). The results are given in table 7 and the recombination percentages based on them are shown in table 10.

TABLE 7

a_l d_p ♀ × "aldpbasp" ♂

1 2 b 3 p_r 4 c_n^2 5 s_p

T°C	0	1	2	3	4	5	1,2	1,3	1,4	2,3	2,4	3,4	1,2,3	1,2,4	2,3,4	T
16°	1093	220	501	69	3	0	17	9	0	15	1	0	0	0	0	
	1085	216	653	95	0	0	13	9	0	15	0	0	0	0	0	4014
25°	1088	190	433	63	2	0	10	7	1	6	0	0	0	0	0	
	1137	213	465	66	5	0	8	2	0	14	3	0	0	0	0	3713
30°	429	63	182	79	10	1	9	10	1	15	4	0	0	1	0	
	434	80	226	65	19	0	11	9	0	14	3	1	1	1	1	1669

Conclusions and discussion

The data supplied by these experiments indicate that the presence or absence of crossing over in one arm of the V-shaped chromosome exerted only negligible influence upon its occurrence in the other. Females bred at high and low temperatures showed the usual temperature effects. The black-purple recombination was about normal (4.2) in the presence of C_R and it rose normally to 11.7 at 30° C and to 5.0 at 16.5° C. Similarly aristaless-dumpy had a recombination percentage of 11.6 at 25° C (in the presence of C_R), 11.1 at 30° C, and 12.0 at 16.5° C. Other similar relations

TABLE 8

a_l t_w L^e s_p

♀ × "aldpbasp" ♂

1 2 b 3 p_r 4 $5v_g$ 6

	14°C	16.5°	25°	30°		14°C	16.5°	25°	30°
0	525+569	813+862	1545+1676	648+671	1,4,5	1+0	0+1	..	1+0
1	63+88	129+172	228+282	129+132	1,4,6	5+5	8+6	9+15	12+10
2	213+273	356+390	544+661	275+312	1,5,6	1+0	2+2	1+3	2+1
3	117+114	98+111	98+97	92+89	2,3,4	2+3	1+3	1+6	1+3
4	138+149	138+152	259+234	170+188	2,3,5	1+1	1+1	0+1	1+0
5	26+43	56+59	101+127	49+57	2,3,6,	9+8	7+7	3+5	11+8
6	214+248	437+410	864+894	334+343	2,4,5	..	1+5	1+2	3+3
1,2	7+8	11+6	13+21	19+14	2,4,6	10+4	13+10	18+22	18+28
1,3	12+8	12+14	10+8	12+24	2,5,6	1+0	1+2	5+4	5+6
1,4	18+28	26+19	34+42	31+35	3,4,5	0+1	0+1	1+2	2+2
1,5	5+6	17+10	27+17	9+10	3,4,6	8+4	1+1	3+1	5+4
1,6	25+41	73+84	107+139	70+90	3,5,6	0+1	..	1+0	2+0
2,3	14+17	11+13	13+7	22+28	4,5,6	1+0	..	0+1	4+2
2,4	44+49	66+82	99+92	59+56	1,2,3,4	..	..	..	1+0
2,5	16+14	26+30	44+41	22+15	1,2,3,5	1+0	..	..	..
2,6	75+98	155+191	288+345	157+165	1,2,3,6	..	2+0	1+0	..
3,4	24+22	14+16	16+11	25+20	1,2,4,5	..	1+0	..	0+1
3,5	10+11	3+10	8+2	6+7	1,2,4,6	..	1+0	1+0	2+1
3,6	29+37	48+55	49+52	57+53	1,2,5,6	..	..	..	1+0
4,5	1+5	5+5	7+5	8+8	1,3,4,6	..	0+1	1+0	0+2
4,6	18+27	16+28	50+66	67+66	1,4,5,6	..	..	..	1+1
5,6	4+4	3+4	10+12	11+14	2,3,4,5	1+0	..	..	..
1,2,3	0+1	1+1	2+0	4+1	2,3,4,6	2+1	..	1+1	2+0
1,2,4	1+0	0+2	1+4	3+8	2,3,5,6	..	..	0+1	0+1
1,2,5	0+0	0+1	1+1	2+1	2,4,5,6	..	..	0+1	2+0
1,2,6	3+1	3+3	5+14	14+15	1,2,3,4,5	..	..	..	1+0
1,3,4	0+3	5+2	2+0	3+2	1,2,3,4,6	..	..	..	2+0
1,3,5	1+1	0+1	1+0	1+2	1,3,4,5,6	..	..	..	1+0
1,3,6	4+6	4+4	5+5	9+13					
					T	3549	5339	9398	4910

are observed in table 10. The values obtained from these experiments demonstrate that high or low temperatures were inoperative as factors bringing about crossing over where it was inhibited by an inversion. The effect of temperature is seen on the regions very close to the spindle fiber which are outside of the inversion, but the cinnabar-2-speck region remained unaffected.

Unfortunately, from the matings made, I cannot draw any conclusions about the possible increase of the frequency of inverse synapsis at the temperatures used.

TABLE 9

	a_1 t_x					L^e s_p			
	1	2 b	3 p_r	4 c_a	5 v_g 6 7	♀ × "aldpbasp" ♂			
	14°C	16.5°	25°	30°		14°C	16.5°	25°	30°
0	416+446	707+710	828+795	589+629	1,6,7	2+0	3+1	1+2	2+3
1	51+66	109+149	126+175	75+120	2,3,4	1+1	1+1	..	3+1
2	207+305	345+464	333+418	267+318	2,3,5	6+5	3+7	1+1	4+3
3	93+90	97+142	77+66	90+91	2,3,6	2+2	1+2	1+0	1+1
4	14+37	30+35	21+33	37+49	2,3,7	12+8	10+4	2+1	4+7
5	103+102	149+174	114+166	96+109	2,4,5	..	1+0	1+0	1+2
6	40+48	74+66	75+50	54+30	2,4,6	0+1	0+2	1+0	1+0
7	215+237	397+398	434+440	317+355	2,4,7	8+3	4+9	2+3	10+6
1,2	8+3	19+16	9+9	12+13	2,5,6	..	1+0	2+1	0+1
1,3	7+8	11+12	6+6	9+14	2,5,7	13+18	13+22	10+6	15+20
1,4	4+7	1+10	6+2	9+8	2,6,7	1+5	2+7	2+2	7+4
1,5	14+14	24+33	18+26	20+19	3,4,5	..	0+1	1+0	0+2
1,6	7+13	13+17	10+11	8+10	3,4,6	..	1+0	..	..
1,7	28+57	48+80	44+86	53+72	3,4,7	0+2	0+2	0+1	2+2
2,3	13+19	26+20	5+5	16+14	3,5,6	..	1+0	..	0+1
2,4	12+16	24+18	10+6	20+24	3,5,7	3+8	9+7	2+3	8+4
2,5	49+84	93+99	56+85	45+67	3,6,7	3+1	1+0	0+1	5+2
2,6	20+29	36+60	24+28	17+29	4,5,6	..	0+1	..	..
2,7	92+115	204+298	168+217	124+195	4,5,7	0+2	1+2	0+1	..
3,4	0+2	4+4	3+0	2+3	4,6,7	..	2+0	..	1+0
3,5	15+15	21+19	14+10	18+16	5,6,7	..	0+1	..	1+0
3,6	8+7	13+10	6+5	7+4	1,2,3,4	..	..	..	1+1
3,7	42+60	58+74	40+39	38+37	1,2,3,6	..	..	..	1+0
4,5	0+3	4+1	3+0	1+6	1,2,3,7	..	1+1	..	..
4,6	2+2	0+1	0+2	1+5	1,2,4,5	..	..	..	1+0
4,7	7+10	10+14	7+7	21+14	1,2,5,7	2+1	3+0	2+1	..
5,6	1+0	2+1	1+6	2+6	1,3,4,5	..	1+0	..	..
5,7	28+41	44+33	25+22	33+34	1,3,4,7	..	..	..	1+1
6,7	2+2	10+6	11+5	8+13	1,3,5,7	0+1	1+0	..	..
1,2,3	..	1+0	2+0	4+0	1,3,6,7	1+0	1+0	..	..
1,2,4	1+1	1+0	0+1	0+1	1,4,6,7	..	..	..	0+1
1,2,5	0+3	1+2	1+1	4+5	1,5,6,7	..	..	..	0+1
1,2,6	2+0	2+2	2+2	0+1	2,3,4,5	..	..	..	0+1
1,2,7	3+5	7+8	0+4	10+11	2,3,4,6	..	..	1+0	..
1,3,4	..	..	1+1	..	2,3,4,7	..	..	..	1+0
1,3,5	4+3	2+5	1+2	5+2	2,3,5,6	..	..	..	0+1
1,3,6	1+0	0+3	0+2	1+1	2,3,5,7	0+3	1+1	..	1+2
1,3,7	7+8	6+9	1+2	2+9	2,3,6,7	..	1+0	..	0+1
1,4,5	1+0	1+0	..	1+0	2,4,5,7	..	1+0	..	0+1
1,4,6	1+0	2+1	..	2+1	2,5,6,7	..	..	1+0	..
1,4,7	..	2+2	2+1	6+2	3,4,6,7	..	..	..	0+1
1,5,6	0+1	..	0+1	1+0	1,2,3,4,7	..	..	..	1+1
1,5,7	6+3	5+5	5+5	2+6	1,2,4,5,6,7	..	..	..	0+1
					T	3521	5739	5284	4514

TABLE 10

Crossing-over values for sections studied.

SECTION	HOMOZYGOUS C_R				NORMAL CHROMOSOME II				HETEROZYGOUS C_R			HOMOZYGOUS C_R, HETER. C_L		
	14°C	16.5°	25°	30°	14°C	16.5°	25°	30°	16.5°	25°	30°	14.5°	25°	30°
a_1–l_a	9.7	10.2 13.1 Average11.7	10.6	15.6 11.3 14.3 Average14.1	9.8	10.8	10.9	11.8 11.8 Average11.8	12.1	11.6	11.1	..	..	..
l_a–b	24.7	25.6 27.0 Average26.3	24.1	27.1 25.1 26.4 Average26.5	30.6	32.2	27.0	29.1 28.3 Average28.8	30.3	25.3	28.0	..	..	..
b–p_r	13.4	9.1 7.7 Average 8.4	4.4	10.8 9.6 10.2 Average10.4	13.0	10.4	5.8	10.3 9.2 Average 9.9	5.3	4.2	11.1	0.8	0.17 0.26 Average 0.2	1.1
p_r–c_a	..	..	..	..	3.9	3.4	2.2	5.8 5.5 Average 5.7	..	..	..	..	..	..
c_a–v_g	..	..	..	..	15.7	13.9	11.2	12.2 13.7 Average12.6	..	..	..	..	..	..
v_g–L^e	..	..	..	..	5.6	6.0	4.8	5.2 5.3 Average 5.3	..	..	..	..	..	..

TABLE 10 (continued)

SECTION	HOMOZYGOUS C_R				NORMAL CHROMOSOME II				HETEROZYGOUS C_R			HOMOZYGOUS C_R, HETER. C_L		
	14°C	16.5°	25°	30°	14°C	16.5°	25°	30°	16.5°	25°	30°	14.5°	25°	30°
L^c-s_p	..	..	..	..	30.2	31.9	30.4	33.4 31.5 Average 32.8	..	..	..	..	..	..
p_r-L^c	16.2	12.3 11.4 Average 11.9	10.7	19.5 15.2 16.0 Average 17.5	..	..	..	..	..	..	..	12.8	11.7 10.9 Average 11.4	14.1
L^c-v_g	4.4	4.5 4.7 Average 4.7	4.6	6.2 5.4 4.6 Average 5.5	..	..	..	..	..	..	..	4.9	4.7 5.4 Average 5.3	5.6
v_g-s_p	25.2	28.5 30.7 Average 29.6	32.0	32.3 33.4 32.4 Average 32.6	..	..	..	..	..	..	..	28.3	33.3 34.2 Average 33.4	37.5
p_r-c_n^2 or p_r-inversion	..	..	..	..	..	..	..	..	0.1	0.3	2.5	..	..	..
c_n^2-s_p or inversion-s_p	..	..	..	..	..	..	..	..	0	0	0.1	..	..	..
a_l-C_y	..	..	..	..	..	..	..	..	..	..	..	0	0	0
C_y-b	..	..	..	..	..	..	..	..	..	..	..	0	0	0

We may compare the inhibiting effects of inversions on crossing over in proximal and in distal regions with respect to the spindle fiber. Since the C_R inversion includes about 25 units, the extent of the normal chromosome between the right end of the inversion and speck is approximately 27.5 units. Nevertheless, only negligible crossing over occurs in this region when C_R is heterozygous, while in the black-purple section, which is only 6 units long, but which differs apparently from the former by its nearness to the spindle fiber, relatively high crossing over occurs. The same is observed for the distance between purple and C_R. To summarize, regions situated near the spindle fiber show crossing over, though reduced, in the presence of an inversion, while much larger regions distal to the inversion show practically none. These facts would lead one to conclude that crossing over (or synapsis) begins at the spindle fiber and not at the ends of the chromosome.

Table 10 shows the purple-cinnabar-2 or purple-C_R recombination percentage to be between 0.1 and 0.27 and to rise at 30° C to 2.4. The black-purple distance rises to 1.1 at 30° C. It is probable that the nearer a section is to the spindle fiber, the greater is its relative rise at high or low temperatures.

EFFECT OF INVERSION UPON THE TEMPERATURE EFFECT AND UPON COINCIDENCE VALUES

Experimental

One object of the following experiments was to observe the behavior of a distal section, normally situated far enough from the spindle fiber so as to show no temperature effect, when it is placed near the spindle fiber by an inversion. The second problem was to investigate variations, if any, in coincidence, that might be produced if the new order of the genes affects the behavior of the chromosomes at crossing over.

In the first series of experiments, females of the constitution black-purple-C_R-vestigial-cinnabar were crossed to males $a_l\ t_z\ L^c\ s_p/a_l\ d_p$. Thoraxate ($t_z$), an allelomorph of dumpy, was used here because homozygous dumpy would fail to show in the presence of vestigial. Although thoraxate is lethal when homozygous, t_z/d_p was found by BRIDGES to be viable. It was easily classifiable under the conditions of these experiments. F_1 females obtained from the above cross, of the constitution $a_l\ t_z\ L^c\ c_n^2\ s_p/b\ p_r\ v_g\ c_n^2$, were backcrossed to males homozygous aristaless-dumpy-black-purple-cinnabar-vestigial-speck ("aldpbasp"). These multiple recessive males were found to be very poorly viable. They mated with difficulty with the F_1 females, which were wild-type in appearance, except for the dominant

Lobe-*c* eye. Large males from first hatches of pair cultures were therefore selected for use. They were placed with the females in $2 \times \frac{1}{2}$ inch vials for two days, after which interval the mated flies were transferred to bottles. On an average one mating in seven was successful. To obtain recombination percentages for the temperatures higher or lower than 25° C, the P_1 flies were allowed to lay eggs at 25° C until larvae appeared in the bottles, which were then transferred to the respective incubators. The F_1 virgin females were isolated from the bottles, which had been kept at the desired temperatures, and were then mated at 25° C, at which temperature they remained.

The results for the first series of experiments, where F_1 females, homozygous for C_R, were mated to aristaless-dumpy-black-purple-cinnabar-vestigial-speck males, are given in table 8. The recombination percentages computed on the basis of the flies obtained in this series of experiments are recorded in table 10.

For the second series of experiments, which were to serve as controls, flies were synthesized to contain the same genes in both normal chromosomes, as in the experimental chromosomes. Females homozygous for black-purple-cinnabar-vestigial were mated to males $a_l\ t_z\ L^2\ s_p/a_l\ d_p$ and the F_1 females were backcrossed to aristaless-dumpy-black-purple-cinnabar-vestigial-speck ("aldpbasp") males. The experiments were performed at the same temperatures as the previous series in which the F_1 females were homozygous for the inversion. In fact the experimental cultures of both series were always kept together in the same incubator at the respective temperatures. The range of variation of all incubators was ± 0.5° C. The results obtained are presented in table 9. In this series, as well as in the previous one, males and females were classified separately because it was believed by Doctor BRIDGES that males having the genes t_z/d_p occasionally fail to show the character, while females show the character strongly. In neither of these experiments did the t_z/d_p male class overlap the wild-type class. Overlap would have given very asymmetrical "1, 2" double crossover classes, which did not occur; nor was the total of the 1, 2 doubles abnormally high. The male and female data have accordingly been combined. The recombination percentages obtained from the different experiments are given in table 10. The standard map of MORGAN, BRIDGES and STURTEVANT in the *Genetics of Drosophila* is reproduced in figure 1 for the purpose of comparison with the values obtained from our experiments. Withal the agreement is quite satisfactory. The fact that the two large sections thoraxate-black and Lobe-*c*-speck do not show close agreement is to be expected, since these distances on the

standard map are built up from their constituents when intermediate genes are used.

Conclusions and discussion of the temperature effect

On comparing the recombination percentages of the flies recorded in tables 8 and 9 which carry inversion bearing and normal chromosomes respectively, we see that both high and low temperatures produce an increase in crossing over which is about twofold for the regions immediately to the right and left of purple, that is, black-purple and purple-cinnabar. The further away a section is from purple the smaller is the influence of high and low temperatures upon crossing over in that section. The most central sections black-purple and purple-cinnabar show an average rise of about 100 percent. In the normal chromosome the distance vestigial-Lobe-*c* is increased only slightly, if at all, and this section is only about 13 units to the right of purple. This gradient of effect by temperature is shown by the normal chromosome and also by the one containing the C_R inversion. (See standard map of chromosome II, Morgan, Bridges and Sturtevant, 1925.)

The rise in the recombination percentage for cinnabar-vestigial in the normal chromosome is variable in quantity though always present. It happens that the value for that interval at 25° C obtained in our experiment (11.2) is higher than the one (9.0) given by MORGAN, BRIDGES and STURTEVANT (1925). The increase in crossing over for c_n–v_g, based on our normal value at 25° C, is 16 percent at 30° C, 27 percent at 16° C and 41 percent at 14° C. The increases for the similarly placed section in the inversion-bearing chromosome, that is, in the purple-Lobe-*c* distance, are 70 percent at 30° C, 11 percent at 16° C and 50 percent at 14° C. The purple-Lobe-*c* distance in the C_R-chromosome consists mostly of genes which were brought into this position by the inversion. Normally this section of the chromosome does not show any special increase in its crossing over at 30° C or 14° C. But in the inversion, purple-Lobe-*c* increases markedly, as stated above. In the normal chromosome the section between purple and vestigial, consisting of purple cinnabar and cinnabar vestigial, increases 45 percent at 30° C, 35 percent at 16° C and 50 percent at 14° C. These figures are quite comparable with those given above for the percentage increase in the identically situated section within the inversion-bearing chromosome.

Attention should be drawn to the fact that the Lobe-*c*-vestigial distance does not show any significant difference in its increase at the effective temperatures in the two chromosomes in which it was studied, the normal and the one bearing the inversion. A comparison of the diagram on page

87 shows that the vestigial-Lobe-*c* section is only slightly nearer to the point of spindle-fiber attachment in the inversion-bearing chromosome than in the normal. The difference in position, about 3 units, was not sufficient to produce a measurable difference in increase in crossing over, especially since the section happens to be situated about eleven units to the right of the spindle fiber, where temperature is not as effective as a modifying agent of crossing over.

Reciprocally, the genic material situated just to the right of the spindle fiber ($p_r - c_n$, $c_n - v_g$) gives a marked increase with the effective temperature when in the normal chromosome. But this same material, when carried by the inversion to a point in the middle of the right arm, gives only negligible increase (part of the $v_g - s_p$ section in the C_R chromosome). Thus three comparisons have been made: (1) Genic material that normally is situated far from the spindle fiber and which there gives only negligible temperature effect gives marked effect when carried near the spindle fiber by the inversion. (2) Genic material that is normally situated near the spindle fiber and there gives a marked temperature effect no longer gives this effect when those genes are placed more distal by the inversion. (3) The genes near the center of the inversion (the $v_g - L^e$ section) are not much displaced nearer to or farther from the spindle fiber by the inversion, and both in the normal chromosome and in the C_R chromosome this section gives the same, not-marked, temperature effect. The conclusion to be drawn from these observations is that the sequence of the genic materials within the chromosome studied is not an essential part of the reactions or mechanisms which are affected by the temperature. The temperature effect is determined by the position of the genes in relation to the spindle fiber and not by the specific nature of the section of the chromosome itself. This confirms the conclusion reached by BRIDGES from study of the relation of the spindle-fiber position to the intensity of the age effect in normal chromosomes.

The next point upon which these experiments were expected to shed some light was the problem of coincidence. By coincidence is meant the ratio of the number of double crossovers obtained to the number expected if one crossover does not influence the position of the next. When a given distance undergoes 10 percent of crossing over and another one 15, it is expected that simultaneous or coincident crossovers occur in a total of 10 percent $\times$ 15 percent = 1.5 percent. Actually it is found that the number obtained is less than that expected. It was stated above that it would be of interest to know whether the values of coincidence were modified by the change of genic content within the chromosome. Coincidence has been held

TABLE 11

Coincidence values for sections in Normal and C_{IIR} chromosome.

GROUP 1. *Both sections situated in same arm. Sections in right arm whose coincidence values are here compared are identically situated in two chromosomes with respect to distance from p_r.*

	1-2		1-3		2-3		4-5 (C_{IIR}) 5-6 (N)		4-6 (C_{IIR}) 5-7 (N)		5-6 (C_{IIR}) 6-7 (N)	
	LEFT-C_{IIR}	LEFT-N	LEFT-C_{IIR}	LEFT-N	LEFT-C_{IIR}	LEFT-N	RIGHT-C_{IIR}	RIGHT-N	RIGHT-C_{IIR}	RIGHT-N	RIGHT-C_{IIR}	RIGHT-N
14°C	0.19	0.10	0.56	0.34	0.31	0.23	0.24	0.13	0.31	0.41	0.20	0.06
16°C	0.10	0.17	0.50	0.36	0.19	0.24	0.35	0.16	0.23	0.30	0.09	0.14
25°C	0.14	0.11	0.39	0.35	0.20	0.12	0.27	0.26	0.36	0.26	0.16	0.20
30°C	0.18	0.16	0.50	0.45	0.37	0.24	0.33	0.35	0.47	0.35	0.28	0.27
Average	0.15	0.13	0.49	0.38	0.27	0.21	0.30	0.22	0.34	0.33	0.18	0.17

GROUP 2. *Coincidence for sections not situated in the same arm.*

	C_{IIR} CHROMOSOME									NORMAL CHROMOSOME								
	1-4	1-5	1-6	2-4	2-5	2-6	3-4	3-5	3-6	1-5	1-6	1-7	2-5	2-6	2-7	3-5	3-6	3-7
14°C	0.92	0.81	0.84	0.66	0.78	0.79	0.71	1.18	0.65	0.52	1.02	0.81	0.79	0.79	0.64	0.42	0.57	0.74
16°C	0.61	0.92	0.85	0.90	0.87	0.83	0.56	0.63	0.78	0.63	0.80	0.64	0.74	0.85	0.84	0.48	0.64	0.68
25°C	0.70	0.96	0.75	0.79	0.83	0.87	0.62	0.58	0.76	0.68	0.75	0.74	0.89	0.76	0.90	0.71	0.75	0.86
30°C	0.55	0.51	0.79	0.50	0.63	0.76	0.50	0.46	0.60	0.67	0.65	0.72	0.67	0.67	0.75	0.60	0.48	0.52
Average	0.69	0.80	0.80	0.71	0.78	0.81	0.60	0.71	0.70	0.60	0.80	0.73	0.77	0.77	0.78	0.55	0.61	0.70

to arise from the assumed internode formation by chromosomes preceding crossing over. If the postulated internode section, or actual length of block of genes transferred by a double crossover, is a purely physical phenomenon, then its size should not be modified by changes in genic content. The relative internode length can be inferred from coincidence values. Thus, the coincidence for all distances in the two types of chromosomes, normal and "inverted," was computed and is recorded in table 11.

Analysis of coincidence data

Coincidence of sections located in different arms of the same chromosome

The data of the four experiments with homozygous C_R and homozygous normal chromosomes seem to indicate, as found in the past, that the further apart two sections are on the chromosome map the greater are their coincidence values. This behavior is observed regardless of whether both sections lie in one arm of the chromosome or in different arms. For our purposes we may compare sections that are relatively near to each other but do not lie within the same arm. Such sections in the C_R chromosome are black-purple in the left and purple-Lobe-*c* or Lobe-*c*-vestigial in the right limb. Similar sections are black-purple and cinnabar-vestigial in the normal one. It is to be expected that if crossing over in one arm is entirely independent of crossing over in the other, the coincidence of two sections located in different arms should vary about a mean of one. Table 11 shows that this is not the case; the values rarely reach one. Even if we take two sections at opposite ends of the chromosome, as far from each other as possible, the coincidence values obtained, while fairly high, do not consistently reach the value of one.

If we divide all coincidence data into two groups (table 11), one consisting of values of sections within the same arm, either right or left, and the other of sections in both arms simultaneously, we notice that the first group gives fairly uniform values, all low. The only temperature which gives consistently higher values is that of 30° C, while 14° C, which has the same effect on crossing over, does not show the same uniform rise. No rise of coincidence values with temperatures is observed in the second group. It can also be seen that group 2 presents on the whole greater variations than group 1. Another point which the data bring out is that for equivalent lengths of map the intervals near the center show higher values than those at the ends. Unfortunately, these data do not contain small sections at the end of the chromosome, so that no exact comparison of coincidence for identical lengths at the outer and inner ends of an arm

can be made on the basis of small sections. It is apparent, nevertheless, that sections 5–6 of group 1 normal chromosome which includes 14 units in one arm very close to the center give a much smaller value than sections 3–5 group 2 (normal) which is 17 units long and includes both arms.

From the data on coincidence of sections located in both arms of the chromosome it is apparent that crossing over in the two arms is not entirely independent. If crossing over in one arm were entirely independent of that of the other, then, theoretically, coincidence should be approximately one, which was not the case. We must therefore assume that although crossing over in one arm is largely independent of the other, some mechanism exists, connected perhaps with the spindle fiber, which interferes with simultaneous crossing over in both.

Comparison of coincidence in normal and inversion-bearing chromosomes

The figures recorded in table 11, group 1, bring out another point about the behavior of the arms of the C_R and the normal chromosomes. The regions involved in the right arm are so arranged that they correspond to regions analogous with respect to their distance from purple, but genetically different. Thus, 4–5 in the C_R chromosome represents coincidence of the purple-Lobe-*c* and Lobe-*c*-vestigial sections, while its analog 5–6 in normal chromosome represents values for the purple-vestigial and vestigial-Lobe-*c* distances. As demonstrated above, purple-Lobe-*c* in the inversion and purple-vestigial in the normal chromosome involve partly different chromosomal material, and the Lobe-*c*-vestigial and vestigial-Lobe-*c* regions in their respective chromosomes involve the same material but in different order. The inversion occurred so that the Lobe-*c*-vestigial fraction remained in about the same position in respect to the spindle fiber, because purple-Lobe-*c* in C_R and purple-vestigial in the normal chromosome are about equal in length, namely 15 and 17 units each, respectively. Thus, a comparison of the coincidence values in the part of the chromosome between purple and speck should demonstrate whether any significant difference in the presumable loop formation of the two chromosomes has occurred. As far as the coincidence data go, they seem to show good agreement between the two chromosomes, section by section. The values for the left end also agree quite well. On the basis of such general comparison it seems permissible to conclude that the underlying mechanism at work in this case is not affected by the sequence of the genic content of the chromosomes.

SUMMARY

1. It is demonstrated that the suppression of crossing over in the right arm of the second chromosome, which was considered to be due to a genetic factor $C_{IIR}C_v$, is the result of an inversion of a part of the chromosome.

2. The inverted section includes the genes for Lobe-*c* and vestigial, and probably for cinnabar, but not purple. It is therefore located near the center of the chromosome. Its maximum length is estimated to be 25 units.

3. The frequency of inverse synapsis of the chromosomes as calculated is unexpectedly high, approximately one-seventh of the cases.

4. No effect was found for the absence or presence of crossing over in one arm of the second chromosome upon its occurrence in the other arm at various temperatures.

5. The increase in crossing over at 30° C, 14° C and 16° C for sections in one arm, when crossing over is inhibited in the other, is the same as in the normal chromosome.

6. A gradient of size of increase in crossing over values, produced by breeding F_1 females at 30° C and 14° C, extends from purple to the right and probably to the left for about 15 units. This gradient operates to the same degree in the chromosomes homozygous for an inversion as in the normal chromosome.

7. When a piece of chromosome situated normally in a section not affected by temperature (the part of the right of Lobe-*c*) is brought near to the spindle fiber by an inversion, it shows the temperature effect characteristic of its new location. When a piece of chromosome normally situated in a part affected by temperature is removed by inversion and relocated in a more distal region, it loses its sensitivity to temperature and behaves as does genic material that is normally situated at that distance from the spindle fiber. The central genes of an inversion are not displaced toward or from the spindle fiber and correspondingly do not change their reaction to temperature. Increase of crossing over with temperature is thus a property of the position of the genes in relation to the spindle fiber and is not a function of the genes.

8. In the presence of an inversion in one arm (heterozygous), crossing over is much more frequent in a section situated between it and the spindle fiber than in one of equal length situated between it and the distal end of the chromosome. On that basis the probable point of the beginning of crossing over (or synapsis) in this V-shaped chromosome is at the spindle fiber.

9. That coincidence of sections located respectively in the two arms of the second chromosome is uniformly below one, shows that crossing over in the two arms is not completely independent.

10. A comparison between the coincidence values obtained for chromosomes homozygous for an inversion and for the normal chromosomes shows that no significant disturbances are created in the mechanism responsible for coincidence upon inverting part of the chromosome. Sections situated similarly with respect to the spindle fiber give the same coincidence values regardless of change in genic material.

11. The coincidence values of two sections located in opposite arms of the second chromosome are higher for equivalent distances than those of sections located in the same arm.

12. When a section of chromosome from the center is introduced into a terminal region no measurable change in crossing over is observed for the transposed section.

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KARL PEARSON, M.A. (CAMBRIDGE), LL.D. (ST. ANDREWS), F.R.S., Galton Professor of National Eugenics in the UNIVERSITY OF LONDON, Director of the BIOMETRIC LABORATORY and the FRANCIS GALTON EUGENICS LABORATORY, was born in London in 1857. He was educated for a career in law and was admitted to the Bar (Inner Temple) in 1882 but was soon diverted to teaching mathematics in a London college. In the early nineties he formulated the name Biometry for the application of statistical methods to the study of variation and correlation in plants and animals. In 1901 in conjunction with GALTON and WELDON he established *Biometrika* and in 1925 the *Annals of Eugenics*. Both of these journals have been published continuously under PEARSON'S editorship since their founding.

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